Autonomic Cardiovascular Control in Children and Adolescents

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Abstract

This thesis investigated the impact of pubertal maturation and sex on cardiovagal baroreflex sensitivity (BRS) and arterial properties of the common carotid artery (CCA), and the relationship between CCA arterial properties and BRS. This thesis also investigated the effect of orthostatic stress on arterial properties of the CCA and carotid sinus (CS), as well as their impact on BRS in response to orthostatic stress.

Children and adolescents between the ages of 8-18 years were examined. To assess pubertal maturation participants were organized into five pubertal groups based on the criteria of Tanner. BRS was assessed by transfer function analysis in the low frequency range (0.05 – 0.15Hz). Pulse pressure (PP) was measured at the CCA (PP_{CCA}) and CS (PP_{CS}) using applanation tonometry, and at the finger (PP_{Finger}) using photoplethysmography. Ultrasound sonography and applanation tonometry were used to determine the distensibility coefficient (DC) at the CCA (DC_{CCA}) and CS (DC_{CS}). A moderate posture stimulus was implemented by passively moving participants into a 50° seated-recumbent (SR) position.

The results demonstrated a sex-by-maturation interaction on BRS ($p= 0.019$). BRS decreased from early- to post-puberty in males ($p<0.01$), and remained unchanged in females. Females demonstrated greater BRS compared to males post-puberty ($p<0.05$). CCA distensibility was not affected by sex or maturation and was not related to BRS. PP_{CS} was greater than PP_{CCA} ($p<0.001$), while PP_{Finger} was greater than both PP_{CCA} ($p<0.001$) and PP_{CS} ($p<0.001$). In response to SR, the relative change in PP_{Finger} was significantly different than the relative change in PP_{CCA} ($p<0.001$) and PP_{CS} ($p<0.001$),

while the relative change between PP_{CCA} and PP_{CS} were not different. Finally, in response to SR there was a significant decrease in DC_{CS} ($p=0.001$), but not DC_{CCA}. The relative change in BRS in response to SR was significantly correlated to the relative change in DC_{CS} ($p=0.004$), but not DC_{CCA}.

The findings demonstrated an important sex-dependent maturation effect on BRS in children and adolescents that was not explained by CCA distensibility. Also, the CS and CCA responded differently to orthostatic stress. The CS was more suitable to evaluate the effect of arterial distensibility on BRS in response to posture change.

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The journey to completing a PhD is not a flat road, but an uphill battle. It takes the support of those who believe in you to carry you through. I was fortunate enough to have someone believe in me and see my true potential, even when I couldn't see it myself, and without whom, completing this would not be possible. You have done more for me than you could ever know and for that, I am truly grateful.

Table of Contents

List of Abbreviations

BF%	body fat percent
BMI	body mass index
BRS	baroreflex sensitivity
CVLM	caudal ventrolateral medulla
CCA	common carotid artery
CO	cardiac output
CS	carotid sinus
DBP	diastolic blood pressure
DC	distensibility coefficient
DC_{CCA}	distensibility coefficient common carotid artery
DC_{CS}	distensibility coefficient carotid sinus
Early	early-pubertal
FFM	fat-free mass
FM	fat mass
Glu	L-glutamate
HF	high Frequency
HR	heart rate
HRV	heart rate variability
Late	late-pubertal
LF	low Frequency
MAP	mean arterial pressure
NMDA	N-methyl-D-aspartate
Peri	peri-pubertal
Post	post-pubertal
PP	pulse pressure
PP_{CCA}	pulse pressure at common carotid artery
PP_{CS}	pulse pressure at carotid sinus
PP_{Finger}	pulse pressure at finger
Pre	pre-pubertal
RVLM	rostral ventrolateral medulla
RRI	R-R interval
SBP	systolic blood pressure
SR	seat recumbent
$Strain_{CCA}$	strain common carotid artery
$Strain_{CS}$	strain carotid sinus
SV	stroke volume
TPR	total peripheral resistance

List of Tables

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of Figures

Chapter 1: Introduction

Cardiovascular disease is the leading cause of death worldwide with an estimated 17.3 million deaths in 2008.[1] It is projected this number will rise to 23.3 million by 2030.[1] Cardiovascular risk factor assessment in childhood and adolescence may provide an important opportunity to understand and prevent future risk of cardiovascular disease development in adults. Autopsy studies have offered evidence that the process of cardiovascular disease development commences at a young age.[2] Exposure to modifiable risk factors in childhood and adolescence is predictive of subclinical markers of cardiovascular disease in adults.[3]

The use of non-invasive measurement techniques has allowed for the assessment of cardiovascular health in children and adolescents, which includes common carotid artery (CCA) intima-media thickness (IMT) and distensibility measurements, and central pulse wave velocity (PWV).[4] These techniques have furthered our understanding of the impact of early exposure to modifiable risk factors. Children and adolescents exposed to modifiable cardiovascular risk factors demonstrate a negative cardiovascular risk profile with a decrease in CCA distensibility, and an increase in IMT and central PWV.[5-8] These findings are critical as these non-invasive measures have been shown to predict cardiovascular morbidity and mortality in adults.[9, 10]

Autonomic cardiovascular control, as assessed by the measure of baroreflex sensitivity (BRS), is another important marker of cardiovascular health. Cardiovagal BRS refers to the ability of the autonomic nervous system, mainly the parasympathetic nervous system, to regulate beat-by-beat alterations in blood pressure via reflex adjustments in heart rate (HR). A reduced BRS is indicative of diminished autonomic function and is

associated with increased cardiovascular morbidity and mortality in adults.[11, 12] Baroreflex function can be further delineated into two components; the mechanical transduction of stretch to an afferent signal and the neural transduction of arterial stretch into a HR response.[13] These components are referred to as the mechanical and neural components, respectively. They are particularly important when assessing diminished baroreflex function. The mechanical component is often quantified using CCA distensibility. Several investigators have quantitatively evaluated the individual components of the baroreflex in relation to age and hypertension in adults and have demonstrated that reductions in BRS are associated with reduced function of both the mechanical and neural component.[14-16] Moreover, baroreflex function is important for blood pressure regulation in response to orthostatic stimuli. Reductions in BRS associated with orthostatic stress have been associated with the mechanical and neural components as well.[14, 17, 18]

Research assessing autonomic function in children and adolescents has seen an increase in recent years. Cardiovagal BRS is reduced in children and adolescents exposed to modifiable cardiovascular risk factors.[19-23] However, there is a lack of understanding of the maturation and development of BRS in children and adolescents. Furthermore, the impact of maturation on arterial distensibility, and the effect of arterial distensibility on BRS at rest and in response to orthostatic stress have been given little attention in children and adolescents. Understanding the maturation and development of BRS is necessary to determine the significance of diminished BRS at a young age, and possible long-lasting effects. Also, identifying the mechanism primarily influencing BRS in this population is critical for targeting interventions.

This thesis will examine autonomic cardiovascular control in children and adolescents by examining BRS. The impact of pubertal maturation on BRS and CCA distensibility will be assessed, as well as the impact of CCA distensibility on BRS at rest and in response to orthostatic stress.

Chapter 2: Review of Literature

2.1 Autonomic Cardiovascular Control

2.1.1 Autonomic Control of Heart Rate

Heart rate (HR) is under the control of the autonomic nervous system, specifically the parasympathetic and sympathetic nervous systems. The parasympathetic nervous system functions to decrease HR, while the sympathetic nervous system functions to increase HR.[24] Therefore, the parasympathetic and sympathetic nervous systems function in an antagonistic manner. Parasympathetic cardiac vagal fibres originate in the medulla oblongata, specifically at the nucleus ambiguus (NA) and dorsal motor nucleus of the vagus, and pass through the head and neck as the vagus nerve.[24, 25] These vagal fibres then synapse with postganglionic fibres near the sinoatrial (SA) node, thus controlling HR.[25-27] When stimulated, the nerve terminals of the postganglionic fibres release acetylcholine which decreases the rate of depolarization of the SA node and ultimately decreases HR. Cardiac sympathetic fibres originate in the upper thoracic and lower cervical segments of the spinal cord. The sympathetic nerve terminals release norepinephrine, which increases the rate of depolarization of the SA node and ultimately increases HR.[25-27]

2.1.2 Autonomic Control of Blood Pressure

The baroreflex arc is a negative feedback control system regulating beat-by-beat variations in blood pressure (BP) through reflex adjustments in R-R interval (RRI) and total peripheral resistance (TPR). An increase in RRI reflects a decrease in HR, and a decrease in RRI reflects an increase in HR. Arterial baroreceptors are stretch-sensitive mechanoreceptors located in the medial-adventitial layers of the carotid sinus and aortic

arch.[28, 29] A branch of the glossopharyngeal nerve (cranial nerve IX), the sinus nerve, carries impulses from the carotid baroreceptors, while small vagal branches carry impulses from the aortic baroreceptors via the vagus nerve (cranial nerve X). These afferent signals converge centrally within the nucleus of the solitary tract (NTS) in the medulla oblongata.[26] Once afferent neural impulses are transmitted and integrated centrally, an efferent response is initiated that alters parasympathetic and sympathetic activity.[25, 27]

An example of baroreflex function occurs when there is a rise in systolic blood pressure (SBP); the artery wall will expand, causing deformation of the baroreceptors and a resultant increase in afferent neuronal firing. This increased firing initiates a reflex mediated increase in parasympathetic nerve activity that increases RRI (decreasing HR), and decreases sympathetic nerve activity to the peripheral vasculature. Conversely, afferent firing is reduced when SBP decreases, resulting in a decrease in parasympathetic nerve activity and an increase in sympathetic nerve activity. In both scenarios, the neural adjustments will affect both the heart and the blood vessels in an effort to return BP to its original set-point pressure.

Cardiovagal BRS refers to the autonomic control of the parasympathetic nervous system on HR in response to acute and continuous perturbations in BP. Due to the rapid nature of the response in RRI (within one cardiac cycle), it is said to be vagally mediated.[30, 31] As well, it comprises an integrative assessment of baroreflex function that can be quantified by relating the change in RRI to a given change in SBP.[12] Hence, BRS can be used as an assessment of autonomic function and refers to the magnitude of

change in RRI for a given change in SBP. In this regard, a greater BRS is associated with better autonomic function.

BRS can be further delineated into its mechanical and neural components. The mechanical component refers to the mechanical transduction of BP into arterial stretch, and can be quantified as the change in arterial diameter for a given change in SBP.[13] The mechanical component represents the vascular properties of the artery and is responsible for baroreceptor activation. This is most often assessed using the CCA due to its ease of imaging with ultrasound sonography compared to the aorta.[13, 15, 17] Furthermore, bilateral carotid sinus (CS) denervation results in chronically elevated BP,[32] while resistant hypertensives implanted with carotid electrical stimulator devices demonstrate reductions BP.[33] These findings demonstrate the importance of carotid baroreceptors in BP control. The second component is the neural component, which is quantified by the change in RRI to a given change in carotid diameter. This component reflects the neural transduction of carotid stretch into vagal outflow.[13]

2.1.2.1 Arterial Baroreflex Pathway

Carotid baroreceptors have been localized to the medial portion of the CS; distal to the bifurcation.[29] The nerve endings of the CS nerve are located within the medial adventitial border. It has been shown that changes in CS diameter produces proportional firing of baroreceptor afferents.[34] The vascular structure of the CS determines the amount of deformation of the baroreceptors in response to arterial pressure changes.[35, 36] A more distensible or elastic artery will expand to smaller changes in pressure, which allows for smaller changes in BP to be detected. This in turn will result in a more tightly regulated BP. Therefore, the transduction of mechanical stretch into neural signaling relies on the

elastic properties of the artery to distend and create mechanical deformation of the vessel wall in response to an increase in arterial BP.[25, 35]

Afferent excitatory signals from the arterial baroreceptors are relayed to the NTS via the glossopharyngeal nerve. These afferent signals elicit reflex adjustments in parasympathetic and sympathetic nervous system activity. Excitatory signals act on the NTS via the neurotransmitter L-glutamate (Glu), which acts on N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors.[25, 37] Baroreceptor excitatory signals projecting onto the NTS result in excitatory signaling of the caudal ventrolateral medulla (CVLM), which in turn results in inhibitory signals to the rostral ventrolateral medulla (RVLM) via GABAergic neurotransmission, effectively causing sympathoinhibition (decreasing sympathetic nervous system activity).[25-27, 38] Concurrently, excitatory signals from the NTS act on the nucleus ambiguus (NA), increasing parasympathetic activity, and ultimately decrease HR. Together, the NA and RVLM neural pathways serve to alter HR and TPR, and are the pathways by which the baroreflex responds to acute perturbations in BP.

2.2 Baroreflex Measurement

A common method of assessing BRS is through the use of pharmacological techniques with vasoactive drugs to create a rise and fall in arterial pressure.[31, 39-41] This technique is known as the modified oxford technique and is commonly used in order to assess integrated BRS, as well as examining the mechanical and neural components of BRS separately.[13, 15] It is considered the 'gold-standard' technique of determining BRS. This method initiates a decrease in BP through the administration of a vasodilator (sodium nitroprusside), which is followed sequentially by a vasoconstrictor agent

(phenylephrine) to increase BP. Average RRI and corresponding SBP, in bins of 1-3 mmHg, are fitted to a linear regression and BRS is determined by the slope of the line.[40, 42, 43]

This drug-induced method also allows for stimulus-response curves to be generated over a large range of BP.[39, 42] An example of a stimulus-response curve is given in Figure 2-1. The threshold of the baroreflex function curve is a plateau in which a decrease in BP no longer results in a decrease in RRI. The saturation is the upper-level plateau in which an increase in SBP no longer results in an increase in RRI. The response range is the difference in max and min RRI response. The centering point is the SBP value that elicits an equal pressor and depressor response. The gain at the centering point is the max gain and is used as an index of BRS. Finally, the operating point is the SBP and corresponding RRI value recorded pre-stimulus.

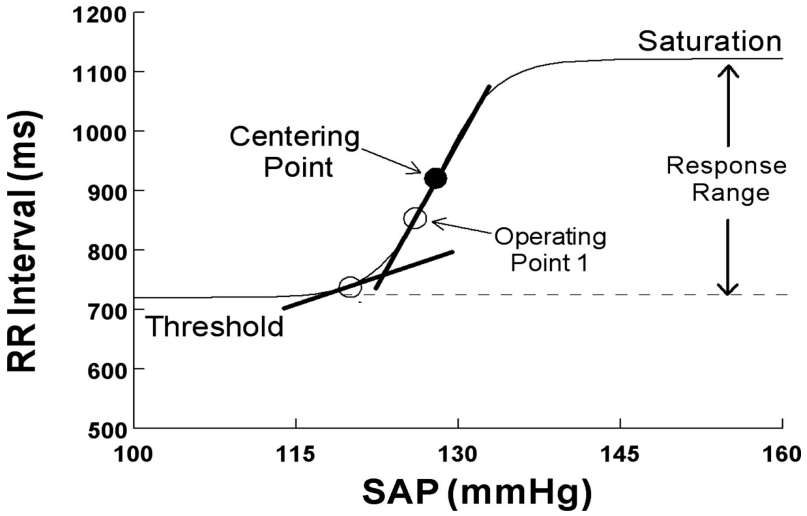

Figure 2-1
Baroreflex stimulus-response curve. Adapted from Schwartz et al., (2013)[42]

This method of assessing BRS is invasive in nature and not always practical in population studies. Furthermore, the invasive nature of drug-induced changes in BRS is not ethically sound for assessment in children. Various non-invasive measurements of

BRS have been implemented such as spontaneous assessments using the sequence technique or transfer function analysis. The non-invasive assessment using transfer function analysis was chosen for this thesis and will be described in detail.

2.2.1 Transfer Function Analysis

Power spectral analysis of beat-by-beat RRI and SBP data is based on the concept that natural spontaneous oscillations in BP elicit oscillations in RRI at a similar frequency range, which is related to baroreflex activity.[12, 44, 45] Two frequency bands are considered: the low-frequency (LF) band (0.05-0.15Hz), and the high frequency (HF) band (0.15-0.5 Hz). Respiratory sinus arrhythmia (RSA) operates within the HF band and is controlled by vagal activity.[46, 47] The baroreflex operates at the LF band and comprises both parasympathetic and sympathetic nervous system activity.[48] In fact, sinoaortic denervation has been shown to eliminate activity in the LF band.[49] Therefore, BRS can be estimated as the LF gain of the transfer function modulus, and is described below.

Successive recordings of SBP and RRI are taken simultaneously and are resampled to become equidistantly spaced in order to create power spectra using fast-fourier transform (FFT).[44, 46] Figure 2-2 (top panel) is an example of a time-series recording of RRI, SBP and diastolic BP (DBP). The bottom panel illustrates the power spectral density of RRI and SBP in their respective frequency domains. Superimposed on the RRI power spectra is a respiratory signal that demonstrates power in the HF band. The power spectra show the amount of variability as a function of frequency, and are calculated as the area under the curve whereby values are expressed as ms^2 for RRI and $mmHg^2$ for SBP.[44, 46, 50] Transfer function analysis is commonly used to examine the gain

of the relationship between SBP and RRI.[44, 46, 47, 51]

The transfer function gain examines the cross-spectral correlation between SBP and RRI power spectra and normalizes it to the power spectrum of SBP. This is calculated in a specified frequency band so long as the linear coupling (coherence) is ≥0.5.[31, 44, 46] The magnitude-squared coherence determines the linear relationship between SBP and RRI in a manner that 0 is considered no relationship and 1 is considered a perfect linear relationship. This technique has been reviewed extensively[12, 45, 52, 53] and applied to both adults,[47, 51, 54] and children and adolescents.[20, 21, 55-59] This technique provides an integrative assessment of cardiovagal BRS.

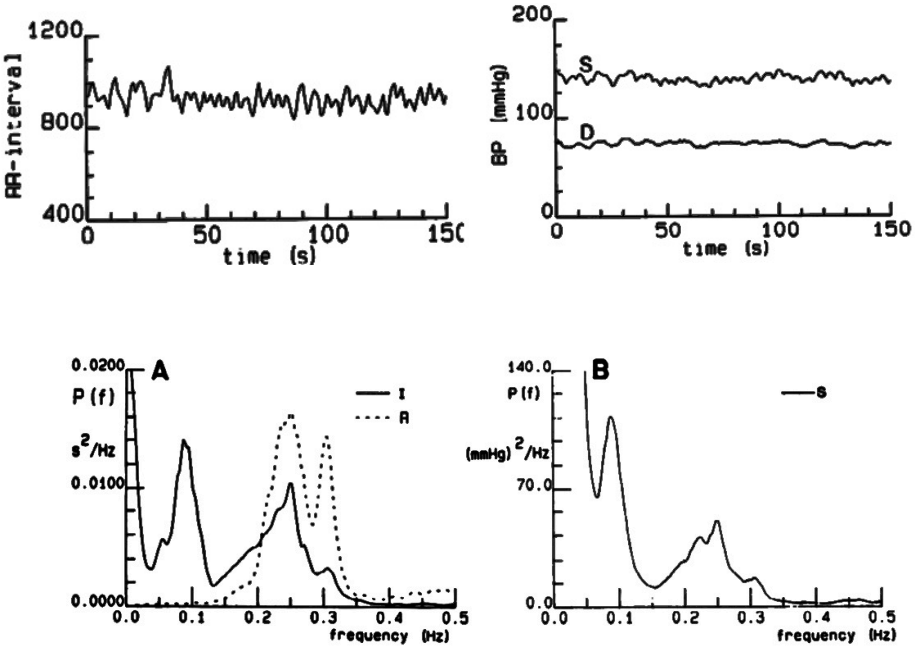

Figure 2-2
(Top) Successive time series collection of RRI (left) and SBP and DBP (right). (Bottom) Power spectra of RRI (left; solid line), respiration (left; dotted line), and SBP (right) represented in their frequency domains. Adapted from deBoer et al., (1987)[46]

Studies have compared BRS assessed by transfer function analysis to pharmacological methods, and demonstrated that it is a useful index of BRS.[42, 44, 49] Robbe et al., (1987)[44] found a correlation of 0.94 ($n=8$, $p<0.001$) during rest, and a

similar correlation was found during task performance, when gain was reduced. These findings suggest that transfer function analysis is a valuable index of BRS at rest and during interventions that reduce BRS. Comparisons with pharmacological techniques cannot be accomplished in children and adolescents due to its invasive nature; however, good agreement has been demonstrated between various non-invasive techniques. Rüdiger et al., (2001)[59] compared transfer function analysis to sequence methods of BRS and found that the sequence method produced significantly greater BRS values, though the correlation between the two methods was strong (r=0.95). Furthermore, a Bland-Altman test showed little difference between the methods.

The use of transfer function analysis in adults has demonstrated good-to-moderate day-to-day reproducibility in healthy adults. The test-retest relative reliability measured in healthy adults demonstrated an intraclass correlation coefficient (ICC) of 0.77 (95% CI: 0.62, 0.87); indicating that 23% of the measurement variability across the study population is due to intraindividual variability, while the remaining is due to interindividual variability.[60] The coefficient of variation in adults has been shown to range from 15-25%. Moderate day-to-day reproducibility of BRS, using transfer function analysis, has also been demonstrated in children and adolescents.[56, 59] Dietrich et al., (2010) demonstrated a moderate intraclass correlation coefficient of 0.49 with a coefficient of variation of 13.8%. Rudiger et al., (2001)[59] found a within session coefficient of variation of 21% in participants between 7-27 years of age. The findings in children and adolescents resemble those in adults. Therefore, the use of transfer function analysis for BRS determination is reliable and sufficient for research purposes to detect real differences between children and adolescents.[56]

The utility and accuracy of the transfer function calculation of BRS has been questioned for its use in examining BRS while resting, or during orthostatic stress.[17, 61] Nevertheless, it has shown to correlate well with the 'gold standard' measure.[42] However, important methodological differences need to be considered when comparing BRS between methods. The modified oxford technique allows for the generation of baroreflex function curves over a large range of SBP values, whereas the transfer function technique examines BRS with more subtle changes in SBP around the operating point.

A recent study by Schwartz et al., (2013)[42] demonstrated that BRS measured at the operating point (21.2 ms/mmHg) using the modified oxford technique provides similar BRS values as those derived by transfer function analysis (19.5 ms/mmHg), compared to 26.1 ms/mmHg derived at the centering point. In fact, all three values corresponded well as the centering point was similar in location to that of the operating point. However, when the centering and operating points are separated as a result of resetting in response to orthostatic stress, the slope at the centering point (28.3 ms/mmHg) becomes significantly different from that of the operating point (8.6 ms/mmHg) for the modified oxford technique. In comparison, BRS measured at the operating point corresponded well with transfer function analysis (8.6 ms/mmHg) in response to orthostatic stress; both demonstrating a 60% decrease in BRS.[42] Most studies using the modified oxford technique derive BRS at the centering point (i.e. maximal slope, G_{max}), not the operating point. Therefore, contrary to previous reports, the use of transfer function analysis for BRS assessment is an accurate assessment of BRS in both supine and HUT.

2.2.2 Measuring the Mechanical and Neural Components of BRS

As previously mentioned, integrated baroreflex function can be separated into its mechanical and neural components. In fact, several investigators have quantitatively evaluated the individual components using both the modified oxford technique and transfer function analysis.[14-16] Using the modified oxford technique, RRI, BP, and carotid artery diameter are recorded simultaneously during the pharmacological increases and decreases in arterial pressure. To examine the mechanical component, systolic carotid diameters and SBP are fitted to a linear regression and the slope of the line is used as an index of the mechanical component.[13] The mechanical component refers to the change in systolic carotid diameter for a given change in SBP. To determine the neural component, RRI is plotted against systolic carotid diameter.[13] The neural component refers to the change in RRI for a given change in systolic carotid diameter.

The mechanical and neural components of integrated BRS have also been assessed using transfer function analysis, as previously described for integrated BRS,[44] by implementing advanced imaging techniques.[14] Simultaneous recordings of carotid arterial diameters and spontaneous arterial BP fluctuations can be measured for extended durations (3-5 mins). The mechanical component can be determined as the transfer function gain of SBP to carotid diameter, while the neural component is the transfer function gain of carotid diameter to RRI.[14]

Together, these techniques have been used to assess the individual components of BRS in a variety of populations and experimental conditions.[14, 15, 17, 62] However, these techniques are difficult to implement in all laboratory settings due to the invasive nature of the modified oxford technique and advanced methodologies required for carotid

diameters for transfer function assessment. Measuring carotid artery distensibility (see section 2.7.3) and its relationship to BRS is a commonly used method to assess the effect of arterial mechanics on BRS; this is particularly useful in an adolescent population. This technique has also been implemented in a variety of populations and experimental conditions.[18, 57, 63, 64] Assessing arterial mechanical properties in relation to BRS is essential as baroreceptor activation occurs in response to arterial distension. Therefore, stimulus detection (pressure change) and baroreflex gain measurements are dependent on the elasticity of barosensory vessels. The buffering capacity of the baroreflex to perturbations in pressure will be reduced in a stiffened vessel compared to an elastic one, as larger changes in pressure are required to provide similar distension. Consequently, evaluation of the neural component of the baroreflex is dependent on the elasticity of the vessel wall and its ability to distend. Moreover, although the aforementioned techniques demonstrate utility for isolating each component of the baroreflex, the neural component is a cluster of indiscernible subcomponents that includes baroreceptor activation and afferent signaling, central processing, efferent fibre activity, and end-organ (cardiac) responsiveness. Taken together, evaluating elastic properties of barosensory vessels and their relationship to baroreflex function provides pertinent information of baroreflex function and dysfunction.

2.3 Clinical Importance of BRS in Adults

The importance of BRS extends beyond its capacity to buffer alterations in BP. It has been well documented in adults that a lower BRS after myocardial infarction increases risk of sudden cardiac death.[11, 65, 66] In a study assessing the clinical implications of BRS in risk stratification of patients with previous myocardial infarctions,

it was found that a BRS of <3 ms/mmHg was a significant and independent predictor of total cardiac mortality with a relative risk of 2.68 (95%CI 1.24-6.16).[11] Furthermore, decreased BRS has been shown to be an independent predictor of cardiovascular mortality in heart failure patients.[67] In this study a BRS value of <3.1 ms/mmHg was associated with a high risk of cardiac mortality (hazard ratio: 3.2, 95%CI 1.7-6).[67] These studies demonstrate the clinical and prognostic importance of measuring BRS in patients with heart failure or a previous myocardial infarction.

Diminished BRS has been demonstrated in aging,[63, 68] as well as in obesity,[69, 70] hypertension,[71] and coronary artery disease,[72] compared to healthy controls; signifying that low BRS is a marker of cardiovascular disease. As mentioned above, this is of clinical importance as reduced cardiovagal BRS is predictive of sudden cardiac death in heart failure patients and individuals with previous myocardial infarction.[11, 67]

The role of baroreflex impairment in children and adolescents is particularly important because cardiovascular health in childhood is predictive of cardiovascular health as an adult.[3] Studies have shown BRS is reduced in children wult with obesity and hypertension.[58, 73] Therefore, assessing autonomic function is clinically important in determining cardiovascular health in children.

2.4 Cardiovascular Risk Factors and BRS in Children

2.4.1 Blood Pressure and BRS in Children

It is important to consider BP levels when assessing BRS as hypertension has been shown to reduce BRS in adults, as well as children and adolescents. Several studies have demonstrated that high BP results in reduced BRS in children and adolescents.[22, 71, 73, 74] Genovesi et al., (2008)[22] found that BRS was reduced in both hypertensive and pre-

hypertensive children compared to controls. Krontoradova et al., (2006)[73] also found that BRS was diminished in children and adolescents with hypertension compared to controls. Likewise, a study by Fitzgibbon et al., (2012)[20] examined the association between BP and BRS in children categorized into high BP and normal BP groups. However, the sample as a whole was not hypertensive, or even pre-hypertensive. It was found that children in the high BP group had significantly reduced BRS compared to those in the normal BP group, even after controlling for sex, BMI, and maturation. This study extends previous findings to a clinically normotensive sample, and further highlights the important relationship between BP and BRS.

Outlining detailed mechanisms associated with reduced BRS with elevated blood pressure is beyond the scope of this study and will only be covered briefly. Hypertension can negatively impact both components of the baroreflex. It has been demonstrated in children and adolescents, as well as adults, that hypertension and elevated BP decreases arterial distensibility.[5, 8, 75, 76] This decrease in distensibility (increased arterial stiffness) may be associated with diminished BRS in hypertension.[75] Furthermore, a diminished neural component, determined using transfer function analysis in adults, has also been attributed to diminished BRS in hypertensive adults.[16]

2.4.2 Obesity and BRS in Children

The effect of obesity on BRS has been examined in children and adolescents. It has been consistently reported that BRS is reduced with obesity.[19, 58, 71, 73, 77] In a study by Lazarova et al., (2009)[77] BRS was reduced in 20 obese adolescents compared to 20 age- and sex- matched controls. An interesting finding in this study was that BRS was stable over three time intervals, recorded every 15-minutes, in the obese group, while varying

significantly in the control group. Furthermore, a negative correlation between BRS and BMI ($r=-0.51$, $p<0.05$) was found in the obese group. These findings are further supported by studies in larger populations, and demonstrate the importance of BMI when examining BRS.[19, 55] Indeed, our lab has demonstrated the influential role of body fat when examining BRS.[21] When comparing children and adolescents with and without developmental coordination disorder, it was found that BRS was reduced in those with developmental coordination disorder and this difference was explained by increased body fat percentage.

A detailed review on the mechanisms of obesity-related reductions in BRS is beyond the scope of this thesis, and will be covered only briefly. Obesity can impact both components of BRS. Our lab has demonstrated that arterial distensibility is reduced in overweight and obese children and adolescents.[23] The reduction in arterial distensibility may result in decreased BRS, as several investigators have reported an important relationship between arterial stiffness and BRS.[63, 64] Obesity may also influence the neural component of BRS, as it has been shown that microinjection of leptin in the NTS of rats impairs BRS.[78]

2.5 Physiological Factors Associated with BRS

2.5.1 Effect of Age on BRS

Several investigators have demonstrated a negative and linear association between BRS and age in adults.[15, 62, 63, 68, 79-81] Elucidating the mechanism involved with the age-related decline in BRS has proven to be more difficult. Changes can occur in either the mechanical or neural component, or both. Monahan et al., (2001)[62] demonstrated that an age-related reduction in BRS was associated with decreased carotid compliance, and that

endurance trained older adults had higher BRS compared to untrained older adults, which was attributed to improved arterial compliance. Several other studies have demonstrated the importance of arterial stiffness on cardiovagal BRS.[14, 64, 82] Conversely, several studies have suggested the contribution of neural dysfunction on reduced BRS with age is most important.[15-17] Hunt et al., (2001)[15] found that BRS decreased with age as a result of decreased neural and mechanical components. However, BRS was greater in physically active older adults compared to sedentary older adults as a result of an increased neural component. Collectively, studies have demonstrated the importance of both the mechanical and neural components on the age-related decline in BRS.[14-16, 62, 63, 83]

In contrast to findings in adults, the effect of age on autonomic control of BP is less clear in children and adolescents. Lenard et al., (2004)[57] assessed the development of cardiovagal autonomic function in a sample of subjects 7-22 years old and found that spontaneous indices of BRS increased from early childhood (7-10 years old) to adolescence (15-18 years old), and remained elevated in young adults (19-22 years old). In contrast, carotid artery distensibility decreased from early childhood to adolescence, with no further decrease in young adults. It was concluded that increased cardiovagal autonomic function with age was a result of improved neural functioning. However, Zavodna et al., (2006)[84] found that BRS was unrelated to age in children and adolescents aged 11-20 years. Discrepancies in findings with respect to age and BRS in children and adolescents may arise from the lack of consideration of the impact of puberty on autonomic control. Further work is necessary to establish the age-related changes in BRS in children and adolescents.

2.5.2 Effect of Sex on BRS

Investigations into the effect of sex on cardiovagal BRS in both children and adolescents, as well as adults, are similar in that some studies have found females to have lower BRS than males,[55, 68, 85] while others have found no difference.[57, 86, 87] Zavodna et al., (2006)[84] found that BRS, assessed using transfer function analysis, was not different between males and females in a sample of 415 participants aged 11-20 years old. This is consistent with an earlier study in children and adolescents 7-22 years old.[57] In contrast, a study by Dietrich et al., (2006)[55] found that BRS, measured using transfer function analysis, was greater in 10-13 year old males compared to females (16.4 ± 9.4 vs. 14.3 ± 8.7 ms/mmHg, respectively). In adults, Beske et al., (2001)[85] assessed sex differences in cardiovagal BRS while controlling for important determinants of BRS such as age, BMI, oral contraceptives, aerobic fitness, and percent body fat. The results demonstrated an independent sex difference in which women had lower cardiovagal BRS compared to men. Percent body fat was marginally related to BRS, and aerobic fitness had no effect; therefore, mechanisms other than body composition or aerobic fitness are responsible for reduced BRS in women. Similarly, a study by Laitinen and colleagues (1998)[68] investigated the effects of age and sex on cardiovagal BRS and found women to have lower BRS compared to men (10.2 ± 1.1 vs. 15.0 ± 1.2 ms/mmHg, respectively). These differences were not explained by plasma hormone concentrations of epinephrine, norepinephrine, insulin, or renin. Furthermore, 24% of middle-aged women and 24% of old women had a BRS value <3ms/mmHg, while none of the middle-aged males had BRS values <3ms/mmHg, and 18% of older men did.

The importance of sex hormones on BRS has also been evaluated. El-Mas et al.

(1998)[88] examined the effect of estrogen replacement on BRS in ovariectomized rats. In this study, three groups were used: a sham-operated group that received a sham surgery, a group that had their ovaries removed, and a group where estrogen was replaced after ovariectomy. The rats treated with estrogen had similar BRS values to the sham-operated rats and both groups had higher values than the ovariectomized rats. A similar study was conducted in male rats that were either sham operated, or castrated with and without testosterone replacement.[89] The bradycardic response to phenylephrine was reduced in castrated rats when compared to sham-operated controls. However, the male rats that were castrated and subjected to testosterone replacement had a similar bradycardic response to phenylephrine as the sham-operated rats. These findings from animal studies suggest that sex hormones influence BRS. Studies have been extended to humans with inconsistent findings. A study by Huikuri and colleagues (1996)[90] provides evidence that estrogen is protective on BRS. In their study, a group of post-menopausal women on hormone replacement therapy were compared to a group of post-menopausal women not on hormone replacement therapy. Those women on hormone replacement therapy had BRS values higher than women not on therapy. Furthermore, women on estrogen replacement therapy had a similar BRS value compared to age-matched men. However, in a study by Hunt et al., (2001),[91] six-months of hormone replacement therapy did not improve cardiovagal BRS in post-menopausal women. In a recent study by Wenner et al., (2013)[92] a group of young healthy women were given a gonadotropin releasing hormone antagonist for 10 days to eliminate ovarian hormone concentrations, and were then supplemented with estrogen. Those women were also split in to a high orthostatic tolerance and low orthostatic tolerance group based on lower body negative pressure

testing. Cardiovagal BRS, assessed by the modified oxford technique, was not improved with estrogen supplementation in the high tolerance group, but was in the low tolerance group. Additionally, it was found that the low tolerance group had less forearm vasoconstrictor responses to lower body negative pressure, which further decreased with estrogen supplementation. The authors concluded that individuals may vary in their sensitivity to estrogen and improved cardiovagal BRS in the low tolerance group may be a compensatory mechanism for decreased vasoconstrictor responses.

Numerous studies have also assessed the effect of ovarian hormones on BRS during the menstrual cycle, and have reported conflicting findings. Minson et al., (2000)[93] found no difference in cardiovagal BRS in the mid-luteal phase of the menstrual cycle, when estrogen and progesterone are elevated, compared to the early follicular phase, when estrogen and progesterone are low. Similarly, Hayashi et al., (2006)[94] found that BRS remained stable throughout all phases of the menstrual cycle. This is consistent with several other studies demonstrating no effect of menstrual phase on BRS.[87, 95] In contrast, Tanaka et al., (2003)[96] examined cardiovagal BRS using the modified oxford technique throughout the menstrual cycle. During the pressor stimulus (increasing SBP), BRS was significantly greater in the pre-ovulatory phase (high estrogen and low progesterone) compared to both the early follicular phase (low estrogen and progesterone), and the mid-luteal phase (high estrogen and progesterone). There was no difference between the early follicular and mid-luteal phase. Furthermore, in response to the pressor stimulus men had greater BRS compared to women in the early follicular phase only. In response to the depressor stimulus (decrease SBP), BRS was greater in the early follicular phase compared to the pre-ovulatory and mid-luteal phase. Men had lower BRS than females in

the early follicular phase only, in response to the depressor stimulus. In addition, BRS significantly correlated with estradiol concentration, for the pressor test only.

It has been suggested that disparities in BRS fluctuation throughout the menstrual cycle may be a result of varying participant age and hormonal concentrations among studies.[92, 94] The method of measuring BRS may also influence findings between studies. In the study by Tanaka et al., (2003)[96] there was no difference in menstrual cycle phase evident using the spontaneous sequence technique for increases in SBP, while only decreases in SBP followed a similar pattern as seen in the depressor response using the modified oxford technique. Furthermore, no sex differences were evident using the spontaneous sequence technique. A similar pattern is evident for studies assessing sex differences in adults, in which spontaneous measurements of BRS do not demonstrate sex differences in BRS.[81, 86, 97]

2.5.3 Maturation

Maturation is the process of attaining biological maturity and is typically evaluated by assessing sexual maturation. Sexual maturity refers to fully functioning reproductive capabilities. Maturation of the nervous and endocrine systems is a major factor underlying sexual maturation.[98] Puberty is a process that involves growth and sexual maturation, resulting from coordinated neuroendocrine alterations, and leads to internal and external changes in primary and secondary sexual characteristics.[99, 100] It is a process that begins in late childhood and early adolescence. The timing and tempo of puberty refers to the age of onset of puberty and the rate of progression through puberty, respectively.[98, 99] Puberty, and the timing and tempo of puberty, can have important implications for physiological outcomes under study.[101] Sexual maturation is associated

with significant increases in testosterone and estrogen, and is typically assessed by the development of secondary sex characteristics; breast and pubic hair for girls, and testes and pubic hair for boys. The most commonly utilized method of staging puberty is the criteria by Tanner.[102, 103] It is important to understand how the physiological and behavioural changes that occur during puberty impact the outcomes under study.

The period of adolescence has been identified as a critical time period for BP monitoring,[104] and a period shown to influence adiposity.[105, 106] Therefore, puberty is a sensitive period when development is vulnerable to external stimuli, as well as internal stimuli such as hormonal changes. As previously mentioned, BRS is decreased in children and adolescents with cardiovascular disease risk factors.[19, 20, 22, 77] Thus, understanding the healthy maturation of baroreflex function during puberty may provide insight into the impact of diminished BRS, associated with cardiovascular disease risk factors, in children and adolescents. For example, one study assessed the impact of puberty on the therapeutic effects of asthma medication.[107] Pubertal stage was found to be associated with the half-life of the medication, and age alone was not sufficient to determine the dosage.[107] These findings highlight the importance of evaluating pubertal maturation when examining physiological health outcomes.

The following sections will summarize what is known in relation to maturation of the autonomic nervous system and BRS. Due to limited research on BRS, a focus is given to autonomic control of HR as assessed by heart-rate variability (HRV).

2.5.3.1 Maturation and Autonomic Control of HR

As previously mentioned, HR is under the control of the sympathetic and parasympathetic nervous systems. Many studies examining the development of the

autonomic nervous system, with respect to age, have utilized HRV as a surrogate for autonomic function. HRV can be determined by calculating the area under the power spectral density curve of RRI for both the LF and HF domain (Figure 2; bottom panel). The LF domain represents sympathetic and parasympathetic activity, while the HF domain represents solely parasympathetic activity.[50] Furthermore, the LF/HF ratio is often used as a surrogate marker of sympathetic activity.[50] Autonomic cardiac control, assessed by HRV, shows development across the span of infancy, childhood, and adolescence. Korkusko et al., (1991)[108] examined HRV across the lifespan from infants 3-months old to older adults 89 years of age. Individuals were grouped from 3-12 months, and then at 5-year intervals after that. This study found that HR was highest in infants 3-12 months of age (mean=125 bpm, SEM = 3.1), with a significant decline to the third decade of life. During the first two decades of life there was a 5-fold increase in HF power, reaching a maximum at 15-19 years of age, declining thereafter. The rate of increase in LF power lags HF, and reaches its max a decade later. These findings suggest the balance between parasympathetic and sympathetic control of the heart, and the magnitude and rate of development, varies with age.

Similarly, Lenard et al., (2004)[57] examined LF and HF power spectra of HR in children grouped into age categories of 7-10 years, 11-14 years, 15-18 years, and 19-22 years. Both HF and LF peaked in the 15-18 year old group, and were significantly greater than the 11-14 year old group only. This difference disappeared in the 19-22 year old group. Several other studies have demonstrated alterations in autonomic cardiac control in children and adolescents with age.[109-112]

In order to comprehensively assess autonomic control of the heart in children, Tanaka et al., (1998)[113] used pharmacological interventions to isolate both the parasympathetic and sympathetic nervous system contributions to changes in HR, and compared these findings to previously reported results in adults.[113] Children were found to have greater vagal tone (as determined by atropine) and augmented responsiveness to adrenoreceptor stimulation of the heart and blood vessels (indicating lower sympathetic activity) compared to adults. Furthermore, complete autonomic blockade of vagal and sympathetic activity demonstrated a negative correlation between HR and age. The authors concluded that vagal predominance is necessary to balance cardiac function as a result of higher non-neuronal cardiac function in children, which is likely attributed to chronotropic automaticity. Similarly, Yamanka et al., (2006)[114] found that in response to active standing, the change in maximum HR increased with age from childhood (6-9 years old) to young adulthood (20-24 years old). This pattern exhibits maturation of vagal activity and sympathetic activation in children and adolescents. This is a consistent finding in children and adolescents in response to active standing.[115, 116] The greatest increase was seen between the 6-9 year old group and the 10-14 year old group. This finding is consistent with previously mentioned reports demonstrating maturation of autonomic cardiac control in children and adolescents. [109-112]

2.5.3.2 Effect of Maturation on BRS

During adolescence, individuals of the same chronological age may not be at the same stage of pubertal maturation. There is a clustering of age ranges within specific stages of puberty.[101] While various studies have assessed the effect of age on BRS, only one has looked at the effect of maturation. Dietrich and colleagues (2006)[55] examined the

influence of puberty on BRS in a large population-based cohort of children aged 10-13 years. Pubertal stage (pre-adolescent vs. adolescent) was not related to BRS in both supine and standing positions. These findings should be interpreted with caution, as children were broadly grouped and a full assessment of maturation was not completed. Additionally, the change in BRS in going from supine to a standing position was greater in the adolescent group compared to the pre-adolescent group. This finding suggests that autonomic adaptation to orthostatic stress is more pronounced in the mature group. This supports the aforementioned studies of increasing HR response in response to orthostatic stress.[114, 116]

Furthermore, while some studies have controlled for pubertal maturation on BRS, [19, 20] this is not common practice. The importance of understanding the effect of pubertal maturation on BRS is based on the fact that puberty is associated with significant physiological changes,[117] and increases in sex hormone concentration.[99, 101] As indicated in the previous section (see section 2.5.2), sex hormones have been shown to influence BRS. Furthermore, altered autonomic maturation in situations of cardiovascular disease risk factors such as obesity,[19, 58] and elevated BP[20, 71] may have important long-lasting consequences. This cannot be adequately determined prior to understanding the maturation of BRS.

2.6 Baroreflex Response to Orthostatic Stress

Orthostatic stress is the gravitational stress placed on the body when a person moves from a supine to upright position. It is a stress encountered daily that the autonomic nervous system, via the baroreflex, must repeatedly adjust to in order to maintain adequate BP. Orthostatic stressors, such as lower body negative pressure

(LBNP) or head-up tilt (HUT), shift blood volume to the lower extremities, decreasing central blood volume. [118-121] Kitano illustrated the cardiovascular responses of both LBNP, at varying levels, and 60° HUT. With increasing levels of LBNP (-20mmHg to -40mmHg) there was a decrease in cardiac filling, cardiac output (CO), and stroke volume (SV). HR also increased significantly from baseline to -40 mmHg; however, mean arterial pressure (MAP) remained unchanged throughout. Similar responses were evident for HUT. Furthermore, brachial and femoral blood flow decreased with each stimulus level, and brachial and femoral vascular resistance increased. These important hemodynamic adjustments that occur in response to an orthostatic stimulus are baroreflex mediated and involve an increase in CO, via elevated HR, and an increase in peripheral vascular resistance.[118, 119, 121-125]

Assessing BRS during orthostasis provides a means to assess the integrity of the baroreflex to maintain BP. BRS has been shown to decrease in response to orthostatic stress.[17, 18, 123, 126] The mechanism behind the decrease in BRS during orthostasis is not well understood. Some investigators suggest the mechanical component of BRS to be reduced,[14, 18] while others maintain that the neural component is the cause.[17] A study by Saeed and colleagues (2009)[14] reported that the reduction in BRS from supine to sitting was due to attenuation of the mechanical component, while the neural component was not affected. Similarly, a study by Steinback et al., (2005)[18] assessed the relationship between carotid distensibility and BRS in response to HUT and found that the change in maximal distensibility of the carotid artery was positively correlated with change in BRS.

In contrast to these previous findings, a study by Taylor et al., (2013)[17] assessed the mechanical and neural components of BRS during supine and active standing using

the modified oxford technique. BRS was significantly reduced during standing as a result of a reduced neural component.[17] The authors suggested disparities in the literature may be due to the difference in methodologies used, and further suggested the use of spontaneous BRS in previous studies[14, 18] to be an inferior method of choice.

Although differences in methodologies used may explain some of the variability in findings, the location at which the mechanical component has been studied may be a factor as well. For example, it is well established that arterial baroreceptors are located in the medial-adventitial layer of the CS.[28] However, most studies use CCA diameter and peripheral pulse pressure (PP) from the finger, rather than directly evaluating the CS, to assess the mechanical component of the arterial baroreflex.[14, 15, 17] This may be a limitation as Steinback et al., (2004)[127] demonstrated a differential response in central PP compared to peripheral PP in response to orthostasis, where change in PP obtained from the finger did not reflect central changes in PP. Another study demonstrated that the CS stiffens sooner than the CCA in older compared to young adults; therefore, the CCA may not reflect changes in the CS.[128] Taken together, directly assessing the CS may provide a more accurate assessment of the mechanical component of BRS.

The cardiovascular response to orthostatic stress in children and adolescents has been examined using passive HUT,[129] active standing,[55, 114-116, 130] and LBNP.[131, 132] Similar to adults, children demonstrate an increase in HR, DBP and TPR, and a decrease in CO and SV, while SBP and MAP remain stable.[56, 114, 116, 129] Furthermore, forearm flow and calf blood flow decrease, while vascular resistance increases with HUT, suggesting intact peripheral vasoconstrictor responses in children 13-19 years of age.[129] When comparing children to young adults, Yamaguchi et al., (1996)[116] found that the HR

response to active standing was lower in children. Similar findings were evident in a study by Yamanaka et al., (2006),[114] suggesting an underdeveloped autonomic nervous system. This coincides with the finding of maturational development of BRS in children and adolescents at rest[57] and in response to active standing.[55] However, few studies have assessed BRS in response to orthostatic stress in children and adolescents. The results of these studies vary with respect to changes in BRS. Dietrich and colleagues have shown that in response to active standing, BRS decreased in boys and girls similarly.[55, 56] Another study assessed BRS in pre-pubertal children in response to LBNP and found that BRS was not significantly reduced at levels of -15, -20, and -25mmHg; however, it was suggested that the stimulus might not have been sufficient to stimulate arterial BRS.[131] To our knowledge there is no study assessing the arterial mechanical component of BRS in response to orthostasis in children and adolescents.

2.7 Assessing Arterial Stiffness

As previously mentioned (see section 2.3), an important component of baroreflex function is the distensibility, or stiffness of an artery. The CCA is most often used when assessing the effect of arterial distensibility on BRS, and the carotid-cardiac baroreflex is often examined in experimental studies due to its ease of access and the importance of carotid baroreceptors on baroreflex function.[32, 64, 122, 133, 134] Several studies have shown that decreased CCA distensibility is associated with decreased BRS.[15, 62, 63] The following sections will outline the surrogate measures of arterial stiffness and how age, maturation, sex, and several cardiovascular risk factors (i.e. obesity, hypertension) influence arterial stiffness in children and adolescents. Lastly, it will explore what is known about arterial stiffness and BRS in children and adolescents.

2.7.1 Arterial Structure and Function

The arterial wall of an artery can be divided into three distinct layers; the intima, media, and adventitia.[135] The intima comprises the endothelium, which lines the arterial lumen. This single layer of epithelial cells is metabolically active and plays an important role in smooth muscle cell function.[136] Adjacent to the endothelium is the internal elastic lamina, which is an elastic structure, composed of elastin and collagen.[135] The medial layer contains collagen and elastin fibers, as well as smooth muscle cells. The smooth muscle cells are responsible for changing arterial diameter and tone by vasoconstriction and relaxation.[136] A surrounding layer of thicker elastin bands, called the external elastic lamina, wraps this layer and provides structural support and protection. The outermost layer of the artery is the adventitia, which is a layer of elastic and collagen that connects the surrounding tissues.[136]

The composition of the arterial wall changes throughout the arterial tree and results in variations in elastic properties along the arterial tree; with large central arteries being more elastic and small peripheral arteries being stiffer.[137] Elastin is the dominant component in the large central arteries, and collagen is dominant in the small peripheral arteries.[136] This is important because collagen has a greater stiffness than elastin.[138] Therefore, the changes in arterial composition create a stiffness gradient in which peripheral arteries are stiffer than the more central large arteries.[137, 139] These structural and functional differences of the arterial segments are important to arterial function; which is to dampen and distribute intermittently ejected blood from the heart into constant steady flow.[137]

The arterial system functions as a conduit to transport oxygenated blood throughout the body, as well as to dampen pulsatile flow from intermittent blood ejection from the heart to steady state flow in order to provide constant tissue perfusion.[140] During systole, when blood is ejected from the heart, a small portion of the SV is propelled forward to the periphery, while a majority is stored in the elastic aorta.[139, 141] The elastic properties of the arterial wall allow the artery to store the energy. During diastole, the stored energy potential is used to recoil the vessel and thus ensuring continuous flow to the periphery.[139, 141] The ability of an artery to accommodate this change in volume is termed compliance, which is the inverse of stiffness.

2.7.2 Local Arterial Stiffness

The arterial wall is considered to be viscoelastic as it contains both viscous and elastic properties.[142] When stress is applied to a perfectly elastic material it will subsequently cause deformation and regain its original form when the stress is removed. However, because the arterial wall has viscous properties, the wall retains some of the deformation caused by the stress. When assessing local arterial stiffness, focus is directed to the elastic properties of the artery due to its ease of measurement.

2.7.3 Measuring Local Arterial Stiffness

The elasticity of the arterial wall can be evaluated by examining the relationship between strain and stress.[142] Stress is considered as the force producing deformation, while strain is the resultant deformation incurred as a percentage change in area. Compliance is considered to be the absolute change in volume (strain) as a result of a change in pressure (stress).[142, 143] This assessment of elasticity is size dependent; meaning a larger artery will appear to be more compliant as it is capable of storing a greater

volume. Distensibility takes into consideration the initial size of the artery, and is the relative change in volume for a given change in pressure. Stiffness is considered to be the inverse of distensibility and compliance. As mentioned, distensibility is a size independent measure of elasticity and will be considered for this thesis. Distensibility can be calculated as the distensibility coefficient (DC) using the following equation[142-144]:

$$DC\ (mmHg^{-1} \times 10^{-3}) = (\Delta CSA) / (PP \cdot CSA_{min}) \qquad (1)$$

Cross-sectional area (CSA) was calculated as $CSA = \pi r^2$, where r = diameter/2 and $\Delta CSA = CSA_{max} - CSA_{min}$. PP is the pressure increase from diastole to systole ($PP = P_s - P_d$). In this context, a greater relative change in CSA for a given change in PP is associated with greater distensibility, and a more elastic artery. A lower distensibility is indicative of a less elastic artery.

Ultrasound is the most commonly used technique to non-invasively assess the arteries in both children and adults.[4, 137] Two-dimensional B-mode images are recorded using a high-frequency linear array transducer.[4] Carotid sonography is completed for the CCA 1-2cm proximal to the bifurcation, and at the proximal portion of the ICA for the CS, just distal to the bifurcation. An example of the change in lumen CSA from diastole to systole is depicted in Figure 2-3.

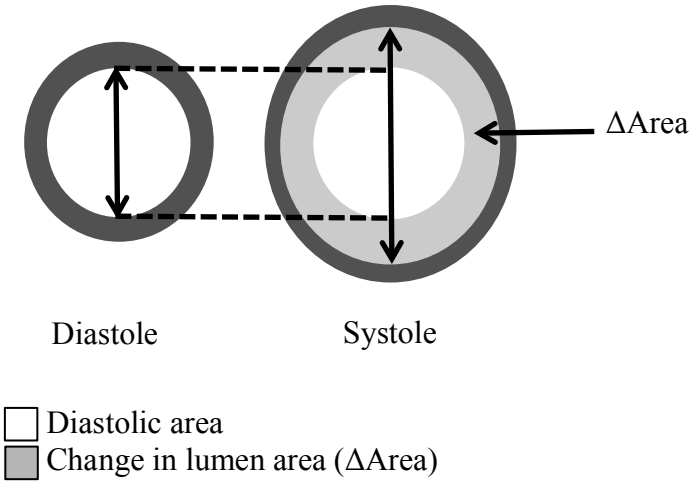

Figure 2-3
Illustration of the measurement of change in cross-sectional area from diastole to systole using ultrasound sonography. Recreated from Laurent et al., (2006)[137]

In addition, simultaneous applanation tonometry is used to non-invasively determine CCA and CS PP. A hand-held tonometer (Millar Instruments, Houston Texas) is used to manually obtain continuous pressure waveforms from the CCA and CS. The pressure wave obtained is very similar to that recorded within the artery. [145, 146] Since the pressure required to applanate the artery and compress the overlying structures varies, the absolute values of systolic and diastolic pressure are not reliable, but the amplitude (PP) can be determined reliably.[127] Accuracy of the Millar devices at the sites of the carotid and radial arteries has been established.[145, 146] Sample ultrasound images and corresponding pressure waveforms for the CCA (Appendix A) and CS (Appendix B) are provided.

2.7.4 Intima-media Thickness

Intima-media thickness (IMT) is a surrogate measure of wall thickness that is quantified by measuring the distance from the lumen-intimal border to the medial-

adventitial border, using high-resolution ultrasound. A sample ultrasound image and IMT measurement is provided in Appendix C. The IMT of the CCA is associated with cardiovascular disease in adults and increased risk of cardiovascular related events.[147-152] Evaluating carotid IMT (cIMT) in children and adolescents demonstrates that cardiovascular risk factors such as obesity and high BP increase cIMT.[153, 154] There also appears to be a weak relationship between age and cIMT in children and adolescents, while sex differences emerge in adolescence.[155-158] cIMT is correlated with body size, BMI, and BP,[155-158] which are known to increase with maturation and growth. The evaluation of cIMT will be used in this study to examine the structural changes that may occur within the CCA with age, maturation, and sex.

2.7.5 Arterial Stiffness in Children and Adolescents

Noninvasive measurement of arterial distensibility is an important tool used to assess cardiovascular risk in children and adolescents.[5, 159] The ability to detect alterations in distensibility and vascular structure has been demonstrated in a variety of pathological conditions in children and adolescents; which include obesity,[5, 6, 23, 160] dyslipidemia,[5, 161] diabetes,[162, 163] hypercholesterolemia,[7] and high BP.[8, 164, 165] These findings identify that negative modifications to arterial structure and function are present early in life. Furthermore, increased arterial stiffness is associated with increased left ventricular mass in children and adolescents,[166] which can translate into accelerated cardiovascular risk in adulthood. In adults, an increase in cIMT, a decrease in CCA distensibility, and an increase in central (aortic) PWV are associated with an increased risk of cardiovascular morbidity and mortality.[159] In children, increased arterial stiffness and increased cIMT are present in those with obesity[23, 167] and hypertension.[8]

There are several important studies in children and adolescents addressing the role of age, sex, and maturation on CCA distensibility and structure. It has been shown that CCA distensibility decreases with aging. This was first highlighted by Jourdan et al., (2005)[156] and extended to a larger population by Doyon et al., (2013).[155] Doyon et al., (2013)[155] studied 1155 children and adolescents aged 6-18 years old. They examined cIMT and distensibility and found cIMT to increase and distensibility to decrease with age, while sex differences were apparent by the age of 15. Distensibility was independently associated with SBP such that increasing SBP decreased distensibility. It is worth noting that the relationship between age and distensibility ($r=-0.24$, $p<0.001$), as well as age and cIMT ($r=0.15$, $p<0.001$) were weak in this large sample population. Nevertheless, an age associated decrease in CCA distensibility has also been reported in a population of 7-22 year olds.[57] Interestingly, a study by Sass and colleagues (1998)[157] found that cIMT and diameter did not increase from age 10 to 18 years, while sex differences in carotid diameter appeared at the age of 14, aligning with the findings of Doyon et al., (2013).[155] Nonetheless, not all studies have report a sex difference in carotid distensibility.[57, 168] It should be noted that PP used to determine distensibility in the aforementioned studies was taken from brachial manual or automated measurements, and as well as using applanation tonometry at the CCA.[155, 156, 169] Although findings are inconsistent, they suggest that age and sex may influence CCA distensibility in children and adolescents and should be considered. Interestingly, studies demonstrating an age and sex effect on arterial parameters often note that this occurs at the age of 15 years, which may further support a possible maturational influence.

There are limited studies assessing the relationship between maturation and arterial properties in children and adolescents. A recent large-scale study by Marlatt et al., (2013)[169] examined the influence of pubertal development on arterial elasticity in children and adolescents. This was the first study to assess arterial properties by organizing participants into pubertal stages based on Tanner criteria. They were grouped into pre-pubertal (Tanner 1), pubertal (Tanner II-IV), and post-pubertal (Tanner V). There was no significant difference in any measure of CCA elasticity across pubertal stages. The authors concluded that accounting for pubertal maturation when reporting vascular data in children and adolescents in unnecessary; however, those findings are in contrast to other reports. A study by Ahimastos et al., (2003)[170] compared central aortic PWV in pre-pubescent boys and girls to post-pubescent boys and girls. Pre-pubescent girls had greater central aortic stiffness than boys, while post-pubescent boys demonstrated an increase in central PWV and girls demonstrated a decrease. This suggests a possible role of maturation on arterial stiffness. In support of this finding, Ayer et al., (2010)[171] demonstrated that pre-pubertal females demonstrated a greater stiffness index than males, as measured by carotid augmentation index.

The discrepancies in findings between studies may be a result of different measurement techniques encompassing different segments of the arterial tree. CCA distensibility is a measure of local arterial stiffness, while PWV[170] is a measure of regional stiffness.[137] Regional measures of stiffness incorporate multiple arterial segments containing varying degrees of stiffness; hence, relating regional and local measurements of stiffness may not be accurate.[137, 142] Aging has been associated with a reversal of the stiffness gradient, as central arteries become stiffer with aging and

cardiovascular risk factors, while peripheral arteries (e.g. brachial) are unchanged.[137, 172, 173] Another explanation may be that the aorta is more susceptible to maturation or age-related changes than the carotid artery. This is highlighted by findings in adults, which have shown that the aorta stiffens to a greater degree than the CCA with aging and cardiovascular risk factors.[173-175] In children, one study demonstrated strong positive correlations between age, height, body mass, and body surface area with aortic CSA (spearman correlation >0.8) using MRI.[176] Furthermore, there was a negative correlation between age and aortic distensibility and descending aorta PWV in males and females. Therefore, the disparities between the findings of Marlatt[169] and Ahimastos[170] may be due to differences in measuring local versus regional stiffness. Further work is necessary to definitively understand the relationship between maturation and arterial elasticity.

2.7.6 Arterial Stiffness and BRS in Children and Adolescents

Limited studies have assessed the effect of arterial stiffness on BRS in children and adolescents. Lenard et al., (2004)[57] found that CCA distensibility decreased with age, while BRS increased. Likewise, Pinter et al., (2007)[177] found that children and adolescents with surgically repaired transposition of the great vessels had decreased CCA distensibility compared to controls, but no difference in BRS. Both studies suggest neural adaptations maintain BRS in the presence of arterial stiffness. However, limited information exists examining the impact of maturation on BRS and arterial stiffness, and the relationship between arterial stiffness, maturation, and BRS in children.

Chapter 3

Rationale

The conceptual framework of the present thesis design, including specific objectives and corresponding studies, is outlined in Figure 3-1. Autonomic control of BP, assessed via BRS, is an important marker of cardiovascular health. Decreased BRS is associated with increased cardiovascular morbidity and mortality in adults. It is known that cardiovascular health in childhood is predictive of cardiovascular health in adulthood, and BRS is an important tool to detect early autonomic dysfunction. Although current research demonstrates BRS is diminished in children and adolescents with cardiovascular risk factors such as obesity and hypertension, there is limited understanding of the development of BRS in children and adolescents. Understanding the development of BRS is necessary in order to understand the significance of abnormally low values that we see in children and adolescents with cardiovascular disease risk factors. Additionally, determining the contribution of the mechanical (arterial) component of the baroreflex to BRS is essential to understanding its development, as the detection of pressure change and the measurement of baroreflex gain depend on the elastic properties of the vessels containing baroreceptors. Thus, examining the relationship between arterial properties and BRS is necessary to understand baroreflex function in children and adolescents.

Specific Objectives

1) To determine the impact of pubertal maturation and sex on BRS development in children and adolescents.

2) To examine the relationship between arterial properties and BRS in response to orthostatic stress by comparing the CCA and CS.

Purposes and Hypotheses

Objective 1

Study 1 – Chapter 4

Purpose: The purpose of this study was to investigate the influence of pubertal maturation on BRS by examining BRS across all stages of pubertal maturation in males and females. It was hypothesized that BRS increases with increasing maturation group such that increasing stages of maturation is associated with an increase in BRS. It was also hypothesized that this pattern is similar between males and females.

Study 2 – Chapter 5

Purpose: The purpose of this study was to determine whether changes in BRS with pubertal maturation were a result of changes in CCA distensibility. The first objective was to determine the effect of pubertal maturation and sex on CCA distensibility, and the second was to examine the relationship between CCA distensibility and BRS. It was hypothesized that CCA distensibility follows a sex-dependent maturation effect similar to the pattern of BRS. Specifically, it was hypothesized that CCA distensibility decreases with pubertal maturation in males from pre- to post-puberty, but remains unchanged, or increases, in females.

Objective 2

Study 3 – Chapter 6

Purpose: The purpose of this study was two-fold, 1) to evaluate the difference in peripheral (finger) and central (CCA and CS) PP in supine, and 2) to evaluate the

differences in response to a postural stimulus at each measurement site. It was hypothesized that peripheral measures of PP do not accurately reflect central measures of PP. Specifically, it was hypothesized that PP measured at the finger is greater than PP at the CCA and CS, and CS PP is greater than CCA PP due to pressure wave amplification. Furthermore, it was hypothesized that peripheral PP will decrease in response to the postural stimulus while central PP remains unchanged.

Study 4 – Chapter 7

Purpose: The purpose of this study was to determine whether distensibility of the CCA and the CS differ at rest and in response to a postural stimulus, and to determine which is a stronger predictor of BRS response to a postural stimulus. It was hypothesized that distensibility will decrease in response to the postural stimulus in both the CCA and CS; however, CS distensibility will be a better predictor of BRS than CCA distensibility.

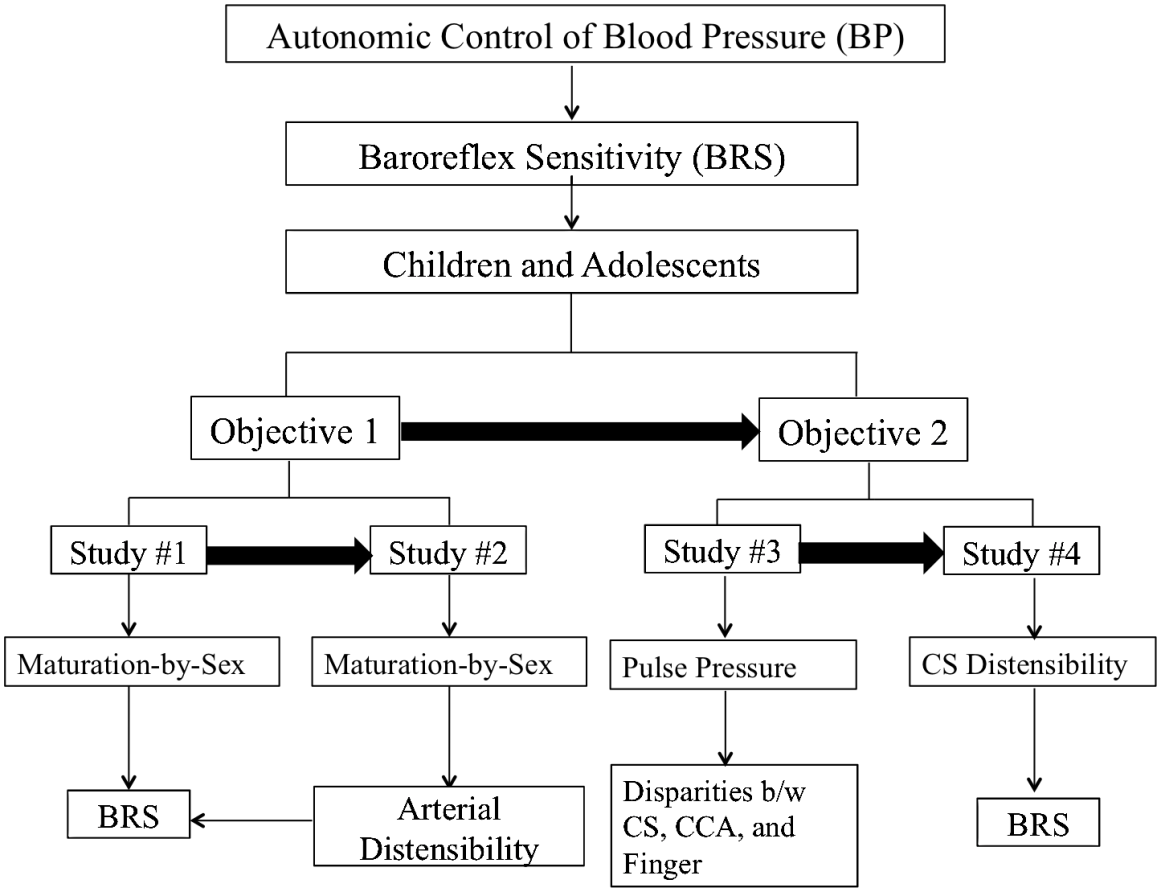

Figure 3-1
Conceptual framework of thesis study design.

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Chapter 4: Study 1

Examining the effects of sex and pubertal maturation on cardiovagal baroreflex sensitivity in children and adolescents

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Abstract

Background: Puberty is associated with important hormonal and metabolic changes associated with growth and maturation. The impact of puberty on blood pressure (BP) regulation remains relatively unexplored. Therefore, the purpose of this study was to examine baroreflex sensitivity (BRS) across different stages of pubertal maturation in healthy children and adolescents.

Methods and Results: The study was cross-sectional and included 104 participants (53 males and 51 females) aged 8-18 years old. Participants were organized into five pubertal groups based on the criteria of Tanner; pre-pubertal (Tanner 1, n=19), early-pubertal (Tanner 2, n=16), peri-pubertal (Tanner 3, n=24), late-pubertal (Tanner 4, n=23), and post-pubertal (Tanner 5 & 6, n=22). Adiposity (fat-free mass, fat mass, and body fat%), body mass index (BMI) and demographic variables were collected. Beat-by-beat BP and R-R interval (RRI) were collected during supine rest in order to determine BRS. BRS was assessed by transfer function analysis in the low frequency range (0.05 – 0.15Hz). The results of the study demonstrated a sex-by-maturation interaction ($F_{(4, 94)}$= 3.202, p= 0.019). BRS decreased from early- to post-puberty in males (30 ± 7.1 vs 13.2 ± 7.8 ms/mmHg), and remained unchanged in females. This led to significantly greater BRS in females compared to males post-puberty (27 ± 7.3 vs 13.2 ± 7.8 ms/mmHg).

Conclusion: Findings from the current study highlight the importance of sex and pubertal maturation on BRS. As well, controlling for both sex and maturation when examining BRS in children and adolescents with cardiovascular disease risk factors will aid in interpreting abnormally high or low BRS values.

Introduction

Arterial baroreceptors are stretch-sensitive mechanoreceptors located in the medial-adventitial layers of the carotid sinus and aortic arch. They play an important role in the short-term regulation of blood pressure (BP). Cardiovagal baroreflex refers to the reflex adjustments in R-R interval (RRI) in response to spontaneous alterations in systolic blood pressure (SBP), such that an increase in SBP will result in an increase in RRI to restore BP.[1] Due to the rapid nature of the response in RRI (within one cardiac cycle) it is said to be vagally mediated. Cardiovagal baroreflex sensitivity (BRS) can be used as an assessment of autonomic function and refers to the magnitude of change in RRI for a given change in SBP.[1] In this regard a greater BRS is associated with enhanced autonomic function. Moreover, cardiovagal autonomic function is an important marker of cardiovascular health as a reduction in BRS is associated with cardiovascular morbidity and mortality.[2-4] Reduced BRS increases risk of sudden cardiac death, and is associated with increased risk of arrhythmias after myocardial infarction, in adults.[2, 3, 5]

Studies have been extended to children and adolescents with cardiovascular disease risk factors and have demonstrated reduced BRS in those with elevated BP[6, 7] and obesity.[8, 9] Although previous studies have found reduced BRS in children and adolescents with cardiovascular risk factors, little is known about the maturation of cardiovagal BRS. Currently, the literature remains equivocal regarding BRS in aging children and adolescents, and whether or not sex differences are present. There is evidence demonstrating that BRS increases[10] or decreases[11, 12] with age in children and adolescents ranging from 7-22 years of age. Moreover, sex differences in children and adolescents have been reported,[12] while others have not demonstrated a difference.[10, 11]

An important point to consider when assessing sex differences in BRS in children and adolescents is the influence of maturation and sex hormones. Evidence suggests that estrogen and testosterone increase BRS in both human (adults)[13-17] and animal[18-20] studies. Given that pubertal maturation is associated with increasing sex hormone concentrations and the timing and tempo of maturation varies between males and females,[21-23] considering pubertal maturation when assessing BRS in children and adolescents is essential. To date, only one study has assessed pubertal maturity on BRS and the results indicated no maturation effect.[12] However, this study did not include a full scope evaluation of pubertal maturity; in fact 80% of the adolescent group (Tanner 2-5) were in early puberty based on the criteria of Tanner. Therefore, the purpose of this study was to examine BRS across different stages of maturation in males and females. It was hypothesized that BRS would increase with maturation similarly between males and females.

Methods

Study Participants

A total of 116 participants were scheduled to participate, whereas 110 completed the study protocol. Of those, 104 (53 males and 51 females) were included in the final sample. Four males and two females were excluded for different reasons. Specifically, two participants (1 male and 1 female) failed to complete the maturation questionnaire correctly, two females were excluded for arrhythmias, and one boy and one girl were excluded for anti-depressant medication. All participants included in the study were healthy children and adolescents aged 8-18 years old with no prior history of chronic illness. The Brock University Research Ethics Board approved this study.

Experimental Protocol

Participants were scheduled for one, two-hour appointment at the Human Hemodynamic Laboratory at Brock University. They were asked to fast four-hours prior to their appointment and to avoid vigorous physical activity, smoking, and caffeine 12-hours prior to their appointment. Upon arrival to the laboratory, the parent/guardian(s) of the participants provided informed written consent if the participant was underage, and the participant provided assent. Information was collected on medical conditions and/or medications. Following this, height and body mass were collected, and adiposity was determined using the BOD POD. Participants then began cardiovascular testing. First, they were asked to lie supine for a period of 15 minutes to ensure BP and heart rate were at baseline levels. Four initial manual BPs were measured and the last three were averaged. Beat-by-beat data collection began and continued for at least five minutes while the subject continued to rest in a dim, quiet, and temperature controlled setting.

Experimental measures

Anthropometry

All measurements of body composition were performed in a private room with the option of having the parent/guardian(s) present. Body mass index (BMI) was calculated using height and body mass (kg/m^2). Standing height (cm) was measured using a stadiometer (Stat 7X, Ellard Instrumentation 50 Ltd Monroe, WA, USA) with the participants' shoes removed and recorded to the nearest 0.1 cm. Body mass (kg) was measured using a digital scale (BWB-800S, Tanita Digital Scale, Tokyo, Japan) and recorded to the nearest 0.1 kg.

Fat mass (FM), fat-free mass (FFM), and body fat percentage (BF%) were determined using whole body air-displacement plethysmography with the BOD POD (Life Measurement, Inc, Concord, CA). The BOD POD is a reliable and valid technique that evaluates body composition in children, adults, and obese individuals.[24] The surface area of hair and clothing has an impact on the measure of air volume[24]; therefore, participants were instructed to wear tight fitting swim suits or spandex and were provided with a Lycra swim cap. The BOD POD was calibrated using a cylinder of known volume.[25] Subjects were then seated in the chamber and body volume measurements were taken twice; each lasting 40 seconds. Subjects were advised to breathe normally and remain relaxed. If both measures were within 150 ml of each other, the mean of the two measures were taken. If the difference between the two measures was greater than 150 ml, a third measure was taken and the mean between the two closest values were used.

Sexual Maturation

Pubic hair development was self-reported using the Sexual Maturation Scale by Tanner criteria, taken from Taylor et al (2001), in order to class subjects into their maturation groups.[26-28] Pubertal maturation was categorized based on self-assessed pubic hair as it has been shown to have better agreement with physical examination by a physician compared to breast/genitalia development.[28] Participants were organized into their maturation groups based on the following: pre-pubertal (Pre, Tanner stage 1), early-pubertal (Early, Tanner stage 2,), peri-pubertal (Peri, Tanner 3), late-pubertal (Late, Tanner 4), and post-pubertal (Post, Tanner 5 & 6).

Blood Pressure

Auscultation is the recommended method of BP measurement in children and adolescents.[29] Participants lied supine with their arm resting at heart level for 15 minutes. Blood pressure was measured four times using a non-invasive, standard inflatable cuff and a sphygmomanometer placed on the right arm. The last three were recorded and averaged to determine SBP, diastolic blood pressure (DBP), and mean arterial pressure (MAP). A stethoscope was placed over the brachial artery pulse, below the bottom edge of the cuff (2 cm above the cubital fossa). Correct measurement of BP requires an appropriate sized cuff; each child was fitted with either a pediatric or adult cuff based on arm size.[29] MAP was calculated using the formula $MAP = 1/3 \cdot SBP + 2/3 \cdot DBP$.

Beat-by-beat RRI and BP

Following 15 minutes of supine rest, five minutes of beat-by-beat RRI and BP data were collected simultaneously. Beat-by-beat BP (SBP and DBP) was collected using photoplethysmography (Nexfin, BMEYE, Amsterdam, The Netherlands) from the middle finger of the left hand. Since BP taken at the finger slightly differs from that taken at the arm, the average manual BPs taken at the beginning of the beat-by-beat recording were used to adjust the beat-by-beat values collected simultaneously with photoplethysmography. RRI was collected using a standard single-lead electrocardiogram. Both BP and RRI were sampled at a rate of 1000 Hz, providing a basic resolution of 1 millisecond (ms). These beat-by-beat data were then used for BRS analysis.

Data Analysis

Cardiovagal BRS

Beat-by-beat data were sampled at 1000 Hz using an online data analysis and acquisition system (Powerlab and Chart 7 PRO, ADInstruments). Data were saved for offline analysis and scanned to ensure that it was free of ectopic beats. Matlab (Mathworks, R2012b) was used to resample the data using the mean cardiac frequency to obtain an equal interval between samples. A low-pass Butterworth filter set to 0.95 Hz was used and the data was detrended. For transfer function analyses, Fast Fourier Transform (FFT) was used with the Welch method and Hanning window, with the window size set to one-fourth of the signal length with one-half overlap. LF area was set to 0.05-0.15 Hz and mean transfer function gain was used to determine BRS for the LF region using a coherence ≥ 0.5.

Statistical Analysis

All statistical analyses were completed using SPSS software (IBM SPSS Statistics 20). Data are expressed as mean ± standard deviation (SD) for continuous variables and as frequencies for categorical variables. The level of significance was set to $p<0.05$ (two-tails).

Descriptive statistics of demographic (age and sex), anthropometric (height, body mass, BMI, FFM, FM, and BF%), and cardiovascular variables (RRI, SBP, DBP, MAP, and BRS) across maturation groups were assessed by a one-way ANOVA and Tukey post-hoc analysis. Independent samples t-tests were used to evaluate descriptive differences between females who reported menarche versus those who did not. Chi-

square tests were used to determine differences in proportions of males and females, maturation group size differences, and their interaction.

Covariates correlated with BRS were identified using Pearson's correlation and significant variables were controlled for in multivariate analyses. Possible covariates included age, height, body mass, BMI, FFM, FM, BF%, BP (SBP, DBP and MAP), and RRI. Two-way ANOVAs were completed for all significant correlates of BRS to determine possible maturation and sex effects, as well as an interaction. A two-way ANCOVA was used to determine the effect of maturation group and sex on BRS, while controlling for previously determined correlates. Each covariate was entered into the model separately in order to conserve power.

All dependent variables were checked for normality using the Shapiro-wilk test, as well as skewness and kurtosis. BRS was not normally distributed for the overall sample. However, there was no significant skewness or kurtosis evident. BRS was normally distributed in all maturation groups except for the Pre group. BRS was normally distributed in both males and females. Levene's test demonstrated homogeneity of variance between maturation groups, as well as between sexes. There were no identifiable outliers in either maturation groups or sexes. Based on the large sample size and visual inspection, the data were deemed acceptable for further parametric analysis.

Results

Descriptive characteristics across maturation

Demographic, anthropometric, and cardiovascular descriptive statistics across pubertal stages are presented in Table 4-1. There was no difference in the distribution of males and females in the total sample (χ^2 =0.038, df=1, ns), or across maturation groups

(χ^2 =1.702, df=4, ns). Furthermore, the number of observations in each maturation group did not differ (χ^2=2.058, df=4, ns). Age increased across all stages of pubertal maturation. Height was significantly greater in the Early group compared to Pre, and increased to the Late group. Body mass was significantly greater in the Peri group compared to Pre and Early, and increased to the Late group. There was no difference in height or body mass between the Late and Post groups. BMI was higher in the Peri group compared to Pre, and Late compared to Pre and Early. There was no further increase in BMI from Late to Post. A total of 15 individuals did not have adiposity assessment due to equipment malfunction and therefore, the analyses for these measures have a sample of 89. FFM significantly increased in the Peri, Late and Post groups. FM was greater in the Late group compared to Pre and Early, and BF% did not change with maturation. SBP was greater in the Late group compared to the Pre and Early groups, and in the Post group compared to Pre, Early, and Peri. DBP did not change with maturation and MAP was significantly greater in the Post group compared to Early. Heart rate was lower in the Post group compared to Pre and Early, as evidenced by lengthening RRI.

In total, 29 of the 51 females (57%) had reached menarche. None of the females in the Pre or Early groups reported menarche, 57% (8/14) reported menarche in the Peri group, 100% in the Late, and 100% in the Post groups. Compared to those who had not reached menarche, menarcheal females were older (15.6 ± 1.8 vs. 11.3 ± 0.85 years, $p<0.001$), taller (164.1 ± 6.7 vs 147.6 ± 7.5 cm, $p<0.001$), had a higher body mass (59.4 ± 10.5 vs. 40.8 ±8.8 kg, $p<0.001$), had greater BMI (22.0 ± 3.5 vs. 18.7 ± 3.5 kg/m^2, $p=0.004$), and higher SBP (115 ± 7 vs 105 ± 9 mmHg, $p=0.001$). There was no difference

in BRS in females who reported menarche vs. those who did not (22.6 ± 8.8 vs. 22.6 ± 10.7 ms/mmHg, ns) and therefore further analysis was not adjusted for menarche.

Table 4-1. Demographic, anthropometric, cardiovascular variables across maturation groups

	Pre	Early	Peri	Late	Post
Age (years)	10.0 ± 1.2	11.5 ± 1.2*	12.9 ± 1.6$^{*\delta}$	14.3 ± 1.8$^{*\delta\tau}$	16.3 ± 1.7$^{*\delta\tau t}$
Sex (Male,Female)	10,9	9,7	10,14	11,12	13,9
Menarche (%)	0	0	57	100	100
Anthropometric					
Height (cm)	140.7 ± 6.4	150.1 ± 9.5*	157.9 ± 10.3$^{*\delta}$	165.6 ± 10.9$^{*\delta\tau}$	170.5 ± 6.7$^{*\delta\tau}$
Body Mass (kg)	35.0 ± 5.4	40.0 ± 8.3	51.4 ± 13.0$^{*\delta}$	60.3 ± 12.4$^{*\delta\tau}$	62.8 ± 9.4$^{*\delta\tau}$
BMI (kg/m^2)	17.6 ± 2.6	17.6 ± 2.5	20.4 ± 3.3*	21.9 ± 3.9$^{*\delta}$	21.6 ± 2.8$^{*\delta}$
Adiposity	n=15	n=12	n=19	n=22	n=21
FFM (kg)	27.7 ± 3.0	33.8 ± 4.8	39.6 ± 9.3*	45.7 ± 9.0$^{*\delta}$	51.8 ± 10.0$^{*\delta\tau}$
FM (kg)	8.0 ± 4.1	7.8 ± 4.5	12.9 ± 8.2	14.3 ± 7.9$^{*\delta}$	10.2 ± 4.5
BF%	21.6 ± 8.2	18.0 ± 7.6	23.6 ± 9.7	24.2 ± 11.2	16.6 ± 7.3
Cardiovascular					
SBP (mmHg)	103 ± 8	104 ± 8	110 ± 8	114 ± 9$^{*\delta}$	117 ± 9$^{*\delta\tau}$
DBP (mmHg)	64 ± 5	61 ± 7	64 ± 8	62 ± 8	65 ± 6
MAP (mmHg)	77 ± 6	75 ± 6	79 ± 6	80 ± 6	82 ± 5$^{\delta}$
RRI (ms)	833 ± 100	875 ± 110	905 ± 144	930 ± 144	1001 ± 141$^{*\delta}$

Mean ± standard deviation. One-way ANOVA with post-hoc Tukey test. Note: $^{*}p<0.05$ compared to Pre-pubertal, $^{\delta}p<0.05$ compared to Early pubertal, $^{\tau}p<0.05$ compared to Peri-pubertal, $^{t}p<0.05$ compared to Late pubertal. BMI = body mass index; FFM = fat-free mass; FM = fat mass; BF% = body fat percent; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; RRI = R-R interval.

Factors associated with BRS

Pearson's correlations for the entire sample (Table 4-2) revealed that BRS was significantly correlated with body mass ($r=-0.232$, $p=0.018$), height ($r=-0.234$, $p=0.017$), SBP ($r=-0.336$, $p<0.001$), and MAP ($r=-0.234$, $p=0.017$). Pearson's correlations were also completed in males and females separately (Table 4-2) and revealed that BRS was significantly correlated with age ($r=-0.387$, $p=0.004$), height ($r=-0.406$, $p=0.003$), body mass ($r=-0.411$, $p=0.002$), BMI ($r=-0.298$, $p=0.03$), SBP ($r=-0.373$, $p=0.006$), and MAP ($r=-0.301$, $p=0.029$) in males. In females, BRS was correlated with RRI ($r=0.334$, $p=0.017$), and a trend was found for SBP ($r=-0.275$, $p=0.051$). Pearson's correlations for measures of adiposity and BRS are presented in Table 4-3. There were no significant correlations between FFM, FM, or BF% with BRS in the total sample ($n=89$) or when split by males ($n=45$) and females ($n=44$).

Table 4-2. Pearson's correlation of baroreflex sensitivity with demographic, anthropometric and cardiovascular variables

	Total Sample n=104		**Males (n=53)**		**Females n=(51)**	
	r	**p**	**r**	**p**	**r**	**p**
Age (years)	-0.188	0.056	**-0.387**	**0.004**	0.005	0.975
Height (cm)	**-0.234**	**0.017**	**-0.406**	**0.003**	0.027	0.850
Body Mass (kg)	**-0.232**	**0.018**	**-0.411**	**0.002**	-0.045	0.756
BMI (kg/m^2)	-0.170	0.084	**-0.298**	**0.030**	-0.093	0.516
SBP (mmHg)	**-0.336**	**<0.001**	**-0.373**	**0.006**	**-0.275**	**0.051**
DBP (mmHg)	-0.073	0.462	-0.113	0.420	-0.024	0.869
MAP (mmHg)	**-0.234**	**0.017**	**-0.301**	**0.029**	-0.147	0.303
RRI (ms)	0.094	0.340	-0.060	0.672	**0.334**	**0.017**

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; RRI = R-R interval.

Table 4-3. Pearson's correlation of baroreflex sensitivity with adiposity

	Total Sample n=89		**Males (n=45)**		**Females n=(44)**	
	r	**p**	**r**	**p**	**r**	**p**
FFM (kg)	-0.200	0.061	-0.249	0.099	-0.060	0.669
FM (kg)	-0.171	0.108	-0.256	0.089	-0.248	0.104
BF%	-0.073	0.499	-0.139	0.361	-0.209	0.174

FFM = fat-free mass; FM = fat mass; BF% = body fat percent**.**

Effect of sex and maturation on correlates of BRS

Two-way ANOVAs were used to examine the influence of sex and maturation on age, height, body mass, BMI, SBP, MAP, and RRI as they were significantly correlated to BRS. Refer to Table 4-1 for between group comparisons for significant maturation effects. Age demonstrated a maturation ($F_{(4, 94)}$=52.133, p<0.001) and sex effect ($F_{(1, 94)}$=8.959, p=0.004), but no interaction ($F_{(4, 94)}$=0.115, ns). Females were older compared to males (13.6 ± 2.4 years vs. 12.8 ± 2.8 years). Height demonstrated a main effect for maturation ($F_{(4, 94)}$ =33.871, p<0.001), but not for sex ($F_{(1, 94)}$=0.008, ns) or their interaction ($F_{(4, 94)}$=2,001, ns). Body mass had a main effect for maturation ($F_{(4, 94)}$ =25.754, p<0.001), but not for sex ($F_{(1, 94)}$=0.317, ns) or their interaction ($F_{(4, 94)}$=0.344, ns). BMI demonstrated a maturation effect ($F_{(4, 94)}$=8.737, p<0.001), but no sex effect ($F_{(1, 94)}$=1.108, ns) or interaction ($F_{(4, 94)}$=0.850, ns). For SBP, a main effect for maturation ($F_{(4, 94)}$=10.27, p<0.001) was found, but not for sex ($F_{(1,94)}$=2.96, ns) or their interaction ($F_{(4, 94)}$ =0.438, ns). MAP demonstrated a maturation effect ($F_{(4,94)}$=10.4, p= 0.002), but no sex ($F_{(1,94)}$=1.874, ns) or interaction effects ($F_{(4,94)}$=0.151, ns). RRI showed a main effect for maturation ($F_{(4, 94)}$=5.065, p= 0.001) and sex ($F_{(1,94)}$=10.4, p=0.002), but not their interaction ($F_{(4, 94)}$=0.686, ns). Females had lower RRI compared to males (872 ± 134 ms vs. 953 ± 136 ms).

Effect of sex and maturation on BRS

A two-way ANOVA was used to evaluate the effect of sex and maturation on BRS. There was no main effect for maturation ($F_{(4, 94)}$=2.0, ns), or sex ($F_{(1, 94)}$=0.704, ns) on BRS, but the sex-by-maturation interaction was significant ($F_{(4, 94)}$=3.202, p= 0.019). Figure 4-1 illustrates the effect of sex and maturation on BRS. BRS decreased from Early

to Post in males (p=0.002), with no change in females, and was reduced in Post males compared to Post females (p= 0.027). The sex-maturation interaction remained after controlling for age ($F_{(1, 93)}$=0.514, ns), height ($F_{(1, 93)}$=0.466, ns), body mass ($F_{(1, 93)}$= 0.969, ns), BMI ($F_{(1,93)}$=0.858, ns), SBP ($F_{(1, 93)}$=4.812, p=0.031), MAP ($F_{(1, 93)}$=2.603, ns), and RRI ($F_{(1,93)}$=3.510, p=0.064) individually.

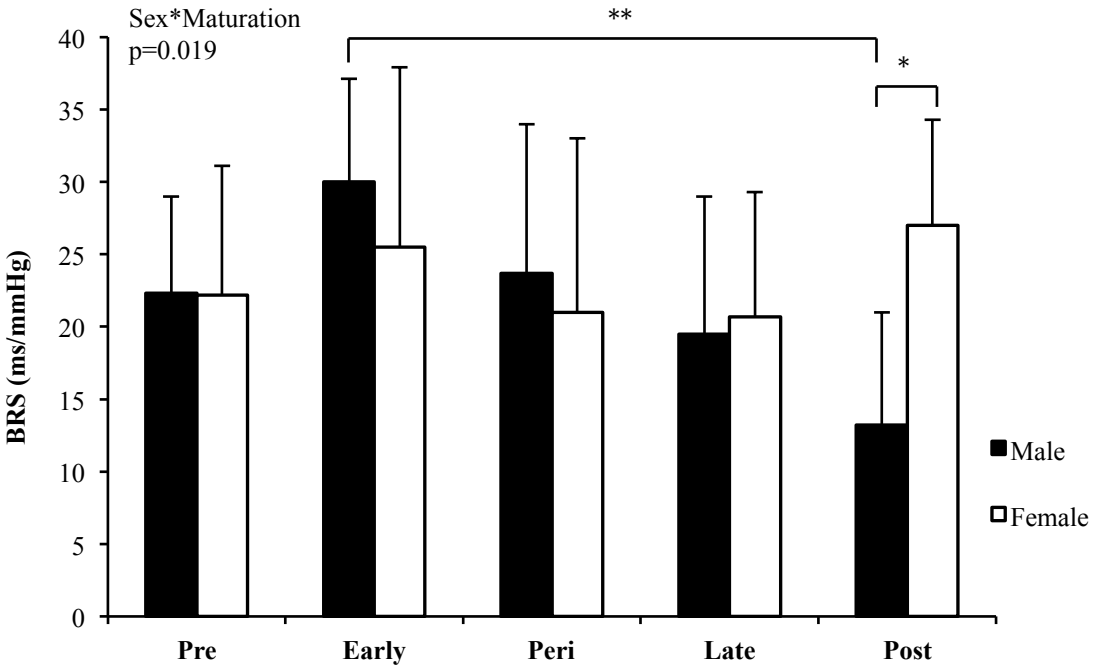

Figure 4- 1
Baroreflex sensitivity (BRS) across maturation groups in males and females. Two-way ANOVA with Tukey post-hoc. Note: *p<0.05, **p<0.01.

Discussion

The novel finding from the present study was a sex-by-maturation interaction in which BRS decreased with maturation from early-to-post puberty in males but remained unchanged in females. Additionally, a sex difference in BRS manifested post-puberty with females having greater BRS. Consistent with previous studies on aging and maturation, height, body mass, BMI, FFM, SBP and RRI increased with maturation.[30-34]

Existing data are equivocal regarding the development of BRS in children and adolescents. Studies have evaluated heart rate variability in childhood showing an increase with age, suggesting autonomic development occurs.[35-37] This is further supported by findings from Lenard and colleagues[10] demonstrating improvements in BRS with increasing age group from 7-18 years, with no further increase to the 18-22 year old group. This improvement in BRS was attributed to improved neural function as arterial distensibility decreased with age. In contrast, Zavodna et al., (2006)[11] found that BRS was not influenced by age in 11-22 year olds. In another study, BRS response to active standing varied with maturation, in which pre-pubertal children demonstrated an attenuated decrease in BRS.[12] However, this study also reported that supine BRS was not affected by maturation. As well, individuals aged 10-13 years old were grouped as either preadolescent (Tanner 1) or adolescent (Tanner 2-5).[12] Disparate findings in previous studies may be a result of evaluating maturation based on age groups,[10] or clustering of children and adolescents at various pubertal stages into one group.[12] This may be problematic as pubertal maturation is associated with physiological changes, in particular, increases in sex hormones. The present study addressed this problem by examining BRS

across five stages of pubertal maturation and found a significant sex-by-maturation effect on BRS.

An interesting finding of this study was that sex differences manifested post-puberty as a result of a significantly diminished BRS in males from the Early to Post puberty. Comparing our BRS values to previous studies is difficult as this is the first to use pubertal grouping by the five stages outlined by Tanner. Our BRS values ranged from 3.5-48.5 ms/mmHg, which is comparable to a previous study that found BRS to range from 1-40 ms/mmHg in individuals aged 11-20 years old.[11] The value for Post males in the present study was similar to reference values of Tank et al., (2000).[38] Their BRS value for those with a mean age of 15 was 14 ms/mmHg, whereas our value was 13.2 ms/mmHg for an average age of 16 years in Post males. Important to keep in mind is that the values from Tank et al., (2000)[38] are not sex specific. As well, correlates of BRS in the present study were similar to those previously identified in children and adolescents.[6, 7, 39] When split by sex, it was apparent that age, height, body mass, BMI, SBP, and MAP were negatively correlated with BRS in males, while RRI was positively correlated with BRS in females. However, these variables did not explain the sex-by-maturation interaction observed in the present study.

The finding of an important maturational sex-based difference in BRS suggests that sex hormones may have an important role in BRS development. Sex hormones were not collected in this study and therefore direct conclusions cannot be made. In adults, few studies have examined the relationship between testosterone and BRS and have demonstrated a positive correlation.[17] Similarly, the beneficial effect of testosterone on BRS has been demonstrated in animal models.[19] These previous studies are limited to

aging adults, adults with heart failure, and animal models, which may not reflect the relationship between testosterone and BRS in adolescents. Furthermore, we found BRS to be greater in females compared to males in the Post group. A beneficial effect of estrogen on BRS has been demonstrated previously in animal studies,[20, 40-42] and throughout the menstrual cycle in women,[15] suggesting a hormonal influence on BRS. Studying sex differences in BRS is complex and has provided equivocal findings. In various studies in adults it has been shown that BRS is greater in males compared to females.[16, 43-46] However, some studies have demonstrated no sex differences in BRS.[14, 47, 48] Furthermore, sex differences seen in young and middle-aged adults are eliminated when comparing older adults.[16, 43, 49] Likewise, studies in children and adolescents remain equivocal on sex differences in BRS, and may be a result of not considering maturation.[10-12] In the absence of data regarding sex hormones and BRS in children and adolescents, further research is required to elucidate the mechanisms responsible for the maturational sex-based difference in BRS identified in the present study.

Another explanation for the changes in BRS with maturation in the present study may be attributed to changes in arterial stiffness. An important component of baroreflex function is the arterial mechanical component, which is distension in response to pressure change, ultimately activating or inactivating baroreceptors to elicit afferent signaling. Several studies have demonstrated the important relationship between arterial distensibility and BRS in adults.[50-55] However, there remain discrepancies surrounding the importance of the arterial mechanical component to changes in BRS.[56-58] Lenard and colleagues (2004) extended this to children and adolescents and found that changes in arterial distensibility and BRS that occur with age were unrelated.[10] Nevertheless, a study

by Ahimastos et al., (2003)[59] demonstrated a sex-by-maturation interaction in central pulse-wave velocity in which females had stiffer arteries pre-puberty, while in post-puberty stiffness decreased in females and increased in males. Therefore, an increase in arterial stiffness with maturation in males may be responsible for the decrease in BRS seen in the present study. In contrast, a recent study has demonstrated carotid artery elasticity is unaffected by maturation.[34] Based on previous conflicting findings future research is necessary to examine the relationship between arterial elasticity, BRS, and pubertal maturation.

Limitations: The current study used self-reported Tanner stage to delineate maturation stage. Although this method has shown good agreement with physician reported maturation stage using Tanner criteria,[28] there may be bias in the self-reported stages, and difficulty in deciphering between stages. Furthermore, the timing and tempo of maturation was not evaluated because Tanner stage was reported on one visit. Therefore, a given group can encompass individuals at the early end, or late end of a given stage. Moreover, hormone concentrations were not collected in this study. However, given the dynamic nature of pubertal development, no criteria are established linking a certain hormone concentration to a given pubertal stage. Therefore, although hormone concentration could have provided further indirect evidence of pubertal maturation, it would not have provided a clear distinction between stages of maturation.

Menstrual cycle phase was not controlled for in the present study. The influence of menstrual cycle phase on BRS has demonstrated inconsistent findings in adults,[15, 58] and has not been evaluated in adolescents. A study by Hayashi et al., (2006)[58] failed to demonstrate variations in BRS throughout the menstrual cycle in young women (20 years

old), while Tanaka et al., (2003)[15] demonstrated a menstrual cycle effect in older women (24 years old). Hayashi suggested the lower concentration of estrogen in younger women might explain the inability to replicate the findings of Tanaka. This rationalization would certainly be applicable to a younger, adolescent population as well. Given the greater menstrual cycle irregularity in adolescent girls and longer intermenstrual cycle length, hormone concentrations are likely to be lower and demonstrate more gradual variations compared to adults. Also, menstrual cycle irregularity increases the complexity of participant scheduling with no previous experimental evidence supporting its importance in adolescents. Therefore, not controlling for menstrual cycle phase in the present study is deemed to be acceptable and not believed to have impacted the results.

The use of spontaneous methods to examine BRS has been criticized as it incorporates both feed forward and feedback mechanisms; however, these methods are positively correlated with pharmacological techniques and provide directionally similar results.[60, 61] Furthermore, this method has been shown to be useful in studying children and adolescents with good reproducibility and is optimal given the invasive nature of other tests that are not ethically measurable in children and adolescents.[11, 62] Lastly, physical activity, which has been shown to improve BRS,[12, 51, 56, 63] was not controlled for in the present study. However, physical activity patterns are not believed to have impacted the results of this study as it has been shown that males spend more time in moderate to vigorous physical activity than females throughout maturation.[64-66]

In summary, this study demonstrated the importance of sex and maturation on BRS in children and adolescents. Males demonstrated a clear decrease in BRS with maturation, whereas females remained unchanged. This was the first study to assess

pubertal maturation and BRS in children and adolescents by categorizing across all stages of sexual maturation. This finding is important to consider when examining children and adolescents with cardiovascular disease risk factors such as hypertension and obesity, as the clustering of maturation in groups may provide errors in interpreting abnormally high or low BRS values.

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Chapter 5: Study 2

The impact of pubertal maturation on carotid distensibility and its relationship with cardiovagal baroreflex sensitivity

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Abstract

Background: Pubertal maturation influences cardiovagal baroreflex sensitivity (BRS) in children and adolescents in a sex-dependent manner. The effect of pubertal maturation on common carotid artery (CCA) distensibility has been given little attention. Furthermore, the relationship between CCA distensibility and BRS in children and adolescents has not been studied extensively. Therefore, we tested the hypothesis that maturational changes in BRS are associated with similar patterns of change in CCA distensibility.
Methods and Results: The study was cross-sectional and included 80 participants (38 males and 42 females) aged 8-18 years old. Participants were organized into five pubertal groups based on the criteria of Tanner; pre-pubertal (Tanner stage 1, n=14), early-pubertal (Tanner stage 2, n=12), peri-pubertal (Tanner 3, n=17), late-pubertal (Tanner 4, n=19), and post-pubertal (Tanner 5 & 6, n=18). Anthropometric (height, body mass, and body mass index) and demographic variables were collected. CCA B-mode imaging and applanation tonometry were used to determine CCA distensibility. Beat-by-beat blood pressure (BP) and R-R interval (RRI) were collected during supine rest in order to determine BRS. BRS was assessed by transfer function analysis in the low frequency range (0.05 – 0.15Hz). The results demonstrated no main effects for sex ($F_{(1, 70)} = 0.462$, ns), or maturation ($F_{(4, 70)} = 0.402$, ns) and no sex-by-maturation interaction ($F_{(4, 70)} = 4.729$, df =4, ns) for CCA distensibility. Furthermore, there was no relationship between CCA distensibility and BRS.
Conclusion: The findings of this study demonstrated that CCA distensibility remains stable throughout pubertal maturation and is not related to BRS. These findings suggest that changes in BRS in children and adolescents are not associated with improved CCA distensibility.

Introduction

The arterial baroreceptors are important regulators of beat-by-beat changes in blood pressure (BP). The integrative assessment of cardiovagal baroreflex sensitivity (BRS) is determined by examining the change in R-R interval (RRI) to a given change in systolic blood pressure (SBP). This integrative assessment can be further delineated into a mechanical and neural component.[1] The transduction of mechanical arterial distension into neural activation is an important determinant of BRS. Increased carotid sinus distension has been shown to activate carotid nerve firing.[2, 3] The neural component encompasses neural transduction of arterial stretch into vagal outflow and a resultant effector response on RRI.[1]

Various studies have examined these components in adults with respect to aging[4-6] and orthostatic stress.[7-9] Monahan et al., (2001)[4] demonstrated that reduced BRS with aging was associated with reduced common carotid artery (CCA) compliance. Furthermore, improvement in BRS as a result of exercise training was associated with improved CCA compliance.[4] Conversely, Hunt and colleagues attributed both the mechanical and neural components to reduced BRS in older adults; however, physically active older adults exhibited preserved BRS as a result of a preserved neural component.[6]

The effect of aging on BRS has been examined in children and adolescents and studies have shown it to increase,[10] or remain unchanged with age.[11, 12] We have demonstrated a maturation effect on BRS that was sex-dependent (Chapter 4). Specifically, it was found that BRS decreased in males from early to post-puberty and remained unchanged in females, which resulted in a sex difference post-puberty. The mechanism of this maturational change has not been elucidated, but is likely a result of

both neural and mechanical alterations associated with maturation.[10, 13] In fact, Lenard and colleagues found that BRS improved with age in children and adolescents aged 7-18 years old.[10] This was attributed to increasing neural mechanisms as CCA distensibility decreased in the same age range.[10]

The effects of age and sex on CCA distensibility has been evaluated in a large cohort of children and adolescents and demonstrated that CCA distensibility decreased with age, while sex differences became apparent at 15 years of age.[14] Similarly, a study examining the influence of maturation on central pulse wave velocity showed that females had stiffer arteries compared to males, pre-puberty.[13] However, stiffness decreased in females and increased in males post-puberty; eliminating sex differences.[13] These studies suggest that sex-dependent maturational changes may affect arterial distensibility; however, one study found that CCA distensibility remained unchanged with pubertal maturation.[15] To date no study has examined the relationship between CCA distensibility and BRS in relation to maturation. Therefore, the purpose of this study was to examine the changes in CCA properties across different stages of maturation, and evaluate the relationship between CCA distensibility and BRS. We tested the hypothesis that sex-dependent changes in BRS with maturation are related to similar sex-dependent changes in CCA distensibility that occurs with maturation.

Methods

Study Participants

There were a total of 121 participants who completed this study, of which, 116 were included from Chapter 4. Participants were between the ages of 8-18 years and reported no prior history of chronic illness. Of these participants, two girls were excluded

for arrhythmias, two participants were excluded for incomplete/incorrect maturation classification, and two were excluded for medication. Of the 115 participants tested in this study, ultrasound images and applanation tonometry for the assessment of the CCA was attainable on 80 participants (~70%). A majority of this dropout was a result of the participants' inability to continue the protocol in order to obtain adequate pressure waveforms and/or CCA images. There was no significant difference between the number of dropouts in each maturation group (χ^2=2.571, df=4, ns). A total of 29% was lost from the pre-pubertal, 14% from the early-pubertal, 23% in the peri-pubertal, 20% in the late-pubertal, and 14% in the post-pubertal groups. Also, to address the relationship between BRS and CCA distensibility, 72 of the 80 participants also had adequate BRS data. There was no difference in age, height, body mass, BMI, SBP, DBP, or RRI between those included in the study and those who were not. The Brock University Research Ethics Board approved this study.

Experimental Protocol

Participants were scheduled for one, two-hour appointment at the Human Hemodynamic Laboratory at Brock University. They were asked to fast four-hours prior to their appointment and to avoid vigorous physical activity, smoking, and caffeine 12-hours prior to their appointment. Upon arrival to the laboratory, the parent/guardian(s) of the participant provided informed written consent if they were underage, and the participant provided assent. Information was collected on medical conditions and/or medications. Following this, height and body mass were collected. Participants then began cardiovascular testing. First, they were asked to lie supine for a period of 15 minutes to allow BP and RRI to achieve baseline levels. Four initial manual BPs were

measured and the last three were averaged. Beat-by-beat data collection of SBP and RRI began and continued for at least five minutes while the subject continued to rest in a dim, quiet, and temperature controlled setting. Following beat-by-beat data collection, carotid ultrasound sonography and tonometry were completed for the CCA.

Experimental measures

Anthropometry

All anthropometric measurements were completed in a private room with the option of having the parent/guardian(s) present. Body mass index (BMI) was calculated using height and body mass (kg/m^2). Standing height (cm) was measured using a stadiometer (Stat 7X, Ellard Instrumentation 50 Ltd Monroe, WA, USA) with the participants' shoes removed and recorded to the nearest 0.1 cm. Body mass (kg) was measured using a digital scale (BWB-800S, Tanita Digital Scale, Tokyo, Japan) and recorded to the nearest 0.1 kg.

Sexual Maturation

Pubic hair development was self-reported using the Sexual Maturation Scale by Tanner criteria, taken from Taylor et al (2001), in order to class subjects into their maturation groups.[16-18] Pubertal maturation was categorized based on self-assessed pubic hair as it has been shown to have better agreement with physical examination by a physician compared to breast/genitalia development.[18] Participants were organized into their maturation groups based on the following: pre-pubertal (Pre, Tanner stage 1), early-pubertal (Early, Tanner stage 2), peri-pubertal (Peri, Tanner 3), late-pubertal (Late, Tanner 4), and post-pubertal (Post, Tanner 5 & 6).

Blood Pressure

Auscultation is the recommended method of BP measurement in children and adolescents.[19] Participants lied supine with their arm resting at heart level for 15 minutes. Blood pressure was measured four times using a non-invasive, standard inflatable cuff and a sphygmomanometer placed on the right arm. The last three were recorded and averaged to determine SBP, diastolic BP (DBP), and mean arterial pressure (MAP). A stethoscope was placed over the brachial artery pulse, below the bottom edge of the cuff (2 cm above the cubital fossa). Correct measurement of BP requires an appropriate sized cuff; each child was fitted with either a pediatric or adult cuff based on arm size.[19]. MAP was calculated using the formula $MAP = 1/3 \cdot SBP + 2/3 \cdot DBP$.

Carotid Sonography and Tonometry

CCA images were obtained using high-resolution ultrasound sonography (Vivid q, GE Medical Systems, The Netherlands). Three 2-dimensional B-mode images were recorded over five cardiac cycles at a frame rate of 37 frames/sec using a 12MHz linear array transducer, for offline analysis. Two of the best quality images with clearly visible intimal layers were used for determining diameter measurements in systole and diastole. Three cardiac cycles were chosen for each of the two images. Carotid sonography was completed for the CCA 1-2cm proximal to the bifurcation. The proper protocols and techniques for standardized carotid ultrasound were employed as recommended.[20, 21]

In addition, applanation tonometry was used to non-invasively determine left CCA pulse pressure (PP). A hand-held tonometer (Millar Instruments, Houston Texas) was used to manually obtain pressure waveforms from the left CCA. The transducer was calibrated with an external device utilizing a two-point calibration system. The pressure

wave obtained is similar to that recorded within the artery.[22, 23] Since the pressure required to applanate the artery and compress the overlying structures varies, the absolute values of systolic and diastolic pressure are not reliable, but the amplitude (PP) can be determined reliably.[24] Accuracy of the Millar devices at the sites of the carotid and radial arteries has been established.[22, 23] In order to obtain an acceptable waveform the criteria outlined by Chen et al., (1996)[23] were followed so that the operator adjusted the hold-down pressure so that acceptable waveforms had a stable baseline, maximum amplitude, and a reasonable configuration. An example of an ultrasound image and corresponding pressure waveforms is provided in Appendix A.

Beat-by-beat RRI and BP

Following 15 minutes of supine rest, five minutes of beat-by-beat RRI and BP data were collected simultaneously. Beat-by-beat BP (SBP and DBP) was collected using photoplethysmography (Nexfin, BMEYE, Amsterdam, The Netherlands) from the middle finger of the left hand. Since BP taken at the finger slightly differs from that taken at the arm, the average manual BPs taken at the beginning of the beat-by-beat recording were used to adjust the beat-by-beat values collected simultaneously with photoplethysmography. RRI was collected using a standard single-lead electrocardiogram. Both BP and RRI were sampled at a rate of 1000 Hz, providing a basic resolution of 1 millisecond (ms). These beat-by-beat data were then used for BRS analysis.

Data Analysis

Cardiovagal BRS

Beat-by-beat data were sampled at a rate of 1000 Hz using an online data analysis and acquisition system (Powerlab and Chart 7 PRO, ADInstruments). Data were saved for offline analysis and scanned to ensure that it was free from ectopic beats. Matlab (Mathworks, R2012b) was used to resample the data using the mean cardiac frequency to obtain an equal interval between samples. A low-pass Butterworth filter set to 0.95 Hz was used and the data were detrended. For transfer function analyses, Fast Fourier Transform (FFT) was used with the Welch method and Hanning window, with the window size set to one-fourth of the signal length with one-half overlap. LF area was set to 0.05-0.15 Hz and mean transfer function gain was used to determine BRS for the LF region using a coherence ≥ 0.5.

Arterial Distensibility

Maximum (systolic) and minimum (diastolic) diameters were determined using semi-automated edge-detection software (Artery Measurement System II, Image and Data Analysis) in a specified region of interest (ROI). The software identifies the artery wall within the ROI based on the contrast of brightness and intensity between the wall and lumen. Lumen diameter (LD) was measured from the leading edge of the near wall intima to the leading edge of the far wall intima. As well, arterial diameter (AD) was measured at the leading edge of the adventitial-medial border of the near wall to the medial-adventitial border of the far wall. Intima-media thickness (IMT) was measured at end-diastole at the far wall for the same cardiac cycles as vessel diameters.

Arterial distensibility coefficient (DC) was calculated for the CCA for both the AD (DC_{AD}) and LD (DC_{LD}) using the standard equation:[25-27]

$$DC\ (mmHg^{-1} \times 10^{-3}) = (\Delta CSA) / (PP \cdot CSA_{min}) \qquad (1)$$

Cross-sectional area (CSA) was calculated as CSA = πr^2, where r = diameter/2 for both AD and LD and $\Delta CSA = CSA_{max} - CSA_{min}$. PP was taken as the pressure increase from diastole to systole ($PP = P_s - P_d$) averaged from 10-15 cardiac cycles using applanation tonometry. As well, distension was calculated as Distension (mm)= $diameter_{max} - diameter_{min}$, and strain was calculated as Strain(%)=$\Delta CSA/CSA_{min}$.

Statistical analyses

All statistical analyses were completed using SPSS software (IBM SPSS Statistics 20). Data are expressed as mean ± standard deviation (SD) for continuous variables and as frequencies for categorical variables. For multivariate analyses, all dependent variables were checked for normal distribution using the Shapiro-wilk test and homogeneity of variances using Levene's test. The level of significance was set to $p<0.05$ (two tails).

Descriptive statistics of demographic, anthropometric, and cardiovascular variables were presented across maturation groups. Differences between maturation groups were assessed by a one-way ANOVA with Tukey post-hoc. Independent samples t-tests were used to compare sex differences for demographic, anthropometric, and cardiovascular variables. This was also repeated for menarcheal status. Chi-square was used to evaluate the distribution of males and females in the total sample and across maturation groups. Pearson's correlation was used to identify correlates of DC_{LD}, DC_{AD}, and IMT.

Two-way ANOVAs were used to determine the effect of maturation group and sex on DC_{LD}, and DC_{AD}, and IMT. Linear regression analysis was used to determine the effect of DC_{LD}, DC_{AD}, and IMT on BRS. As we have shown previously, BRS is affected by sex and maturation; multiple linear regression analyses assessed the effect of CCA distensibility on BRS, while controlling for sex and maturation.

DC_{LD}, DC_{AD}, and IMT were not normally distributed in the total sample or when split by sex. All variables were normally distributed in each pubertal stage except for DC_{LD} and DC_{AD} in the Late group. All variables demonstrated homogeneity of variances based on Levene's test for sex and maturation. Upon visual inspection all variables were deemed acceptable for multivariate testing given the large sample size. Furthermore, log-transformation and non-parametric testing did not change the results.

Results

Baseline Characteristics

The sample included 80 children and adolescents comprised of 38 males and 42 females. There was no difference in the number of males and females in this study for the total sample (χ^2= 0.200, df=1, ns) or across maturation groups (χ^2= 3.176 df=4, ns). Table 5-1 presents descriptive statistics across maturation groups. Age was not different between the Pre and Early groups, but differences became apparent in the Peri group, with age continuing to increase to Post. A similar pattern was evident for height, and body mass. BMI was elevated in the Late and Post groups compared to Early and Pre. SBP was higher in the Late compared to the Pre and Early groups, and Post compared to the Pre, Early, and Peri groups. MAP was greater in Post compared to Early only and RRI was greater in the Post compared to Pre. There was no difference in DBP.

The proportion of girls with menarche varied between groups (χ^2= 28.642 df=4, p<0.001). In total, 23 of the 42 girls (55%) had reached menarche. None of the girls in the Pre or Early groups reported menarche, 60% of girls in the Peri, 91% in the Late, and 100% in the Post groups reported menarche. Menarcheal girls were older (15.6 ± 1.8 vs. 11.3 ± 0.85 years, p<0.001), taller (164.1 ± 6.7 vs. 147.6 ± 7.5 cm, p<0.001), had a greater body mass (59.4 ± 10.5 vs. 40.8 ± 8.8 kg, p<0.001), had greater BMI (22.0 ± 3.5 vs. 18.7 ± 3.5 kg/m^2, p=0.004), had greater RRI (902 ± 120 vs. 795 ± 98 ms, p=0.004), and higher SBP (115 ± 7 vs. 105 ± 9 mmHg, p=0.001) compared to girls who had not reached menarche.

Table 5-1. Descriptive statistics of demographic, anthropometric, and cardiovascular variables across maturation groups

	Pre	Early	Peri	Late	Post
Age (years)	10.2 ± 1.2	11.1 ± 1.1	13.1 ± 1.7$^{**\#}$	14.6 ± 2.0^{**Ŧ}	16.3 ± 1.8^{**Ŧδt}
Sex (M, F)	5,9	7,5	7,10	8,11	11,7
Menarche (%)	0	0	60	91	100
Height (cm)	142.0 ± 6.3	146.3 ± 9.4	156.6 ± 10.8$^{**\#}$	165.3 ± 11.7 ^{**Ŧ}	170.4 ± 7.2^{**Ŧδ}
Body Mass (kg)	35.6 ± 7.1	37.9 ± 6.1	48.8 ± 11.2$^{*\#}$	58.9 ± 11.6^{**Ŧτ}	63.2 ± 9.2^{**Ŧδ}
BMI (kg/m^2)	17.6 ± 3.1	17.7 ± 2.4	19.6 ± 2.7	21.5 ± 3.6$^{*\#}$	21.7 ± 2.5$^{*\#}$
SBP (mmHg)	103 ± 9	104 ± 7	109 ± 9	114 ± 8$^{**\#}$	119 ± 7^{**Ŧτ}
DBP (mmHg)	65 ± 9	61 ± 7	63 ± 7	63 ± 8	65 ± 6
MAP (mmHg)	77 ± 8	75 ± 5	78 ± 6	80 ± 6	83 ± 4$^{\#}$
RRI (ms)	825 ± 121	889 ± 105	907 ± 146	894 ± 140	978 ± 113*

Values presented as mean ± standard deviation. One-way ANOVA with post-hoc Tukey used for between groups comparisons. Pre=pre-pubertal; Early=early-pubertal; Peri=peri-pubertal; Late=late-pubertal; Post=post-pubertal; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; RRI=R-R interval. Note: $p<0.05$ *compared to pre-pubertal; $^{\#}$compared to early-pubertal; ^{τ}compared to peri-pubertal; ŧ compared to late-pubertal. For $p<0.001$ **compared to re-pubertal, ^{Ŧ}compared to early-pubertal, ^{δ}compared to peri-pubertal.

Common carotid arterial properties are presented in Table 5-2. There were no differences between maturation groups in arterial properties. When evaluating sex differences males had greater max LD (Figure 5-1A; 6.35 ± 0.43 mm vs. 6.01 ± 0.49 mm, p=0.002), min LD (Figure 5-1B; 5.45 ± 0.44 mm vs. 5.20 ± 0.45 mm, p=0.013), max AD (Figure 5-1A; 6.96 ± 0.44 mm vs. 6.58 ± 0.51 mm, p=0.001), min AD (Figure 5-1B; 6.12 ± 0.45 mm vs. 5.80 ± 0.46 mm, p=0.002), IMT (Figure 5-2; 0.38 ± 0.048 mm vs. 0.34 ± 0.045 mm, p=0.001), and greater $Distension_{LD}$ (Figure 5-3; 0.89 ± 0.16 mm vs. 0.82 ± 0.15 mm, p=0.035). There was no sex difference for PP (30 ± 9 mmHg vs. 29 ± 7 mmHg, ns). $Distension_{AD}$ (0.84 ± 0.17 mm vs. 0.79 ± 0.16 mm, ns), $Strain_{LD}$ (36.2 ± 8.6% vs. 34.1 ± 8.1%, ns), $Strain_{AD}$ (30.0 ± 7.5% vs. 29.2 ± 7.1%, ns), DC_{LD} (12.8 ± 4.1 $mmHg^{-1}$ x 10^{-3} vs. 12.3 ± 3.6 $mmHg^{-1}$ x 10^{-3}, ns) and DC_{AD} (10.6 ± 3.4 $mmHg^{-1}$ x 10^{-3} vs. 10.5 ± 3.2 $mmHg^{-1}$ x 10^{-3}, ns) were not different between males and females, respectively.

Table 5-2. Arterial properties of the common carotid artery across maturation groups

	Pre	Early	Peri	Late	Post
Common PP	29 ± 6	28 ± 4	29 ± 10	29 ± 7	31 ± 9
LD max (mm)	5.91 ± 0.53	6.16 ± 0.34	6.11 ± 0.49	6.28 ± 0.51	6.31 ± 0.49
LD min (mm)	5.10 ± 0.41	5.34 ± 0.32	5.24 ± 0.52	5.43 ± 0.47	5.41 ± 0.48
AD max (mm)	6.46 ± 0.53	6.71 ± 0.34	6.72 ± 0.52	6.89 ± 0.52	6.93 ± 0.51
AD min (mm)	5.69 ± 0.41	5.94 ± 0.33	5.87 ± 0.53	6.08 ± 0.46	6.08 ± 0.52
$Distension_{LD}$	0.81 ± 0.17	0.82 ± 0.11	0.86 ± 0.18	0.87 ± 0.17	0.90 ± 0.15
$Distension_{AD}$	0.77 ± 0.19	0.77 ± 0.14	0.83 ± 0.18	0.83 ± 0.18	0.85 ± 0.14
$Strain_{LD}$ (%)	34.2 ± 7.4	33.5 ± 6.5	36.8 ± 10.9	34.2 ± 8.3	36.3 ± 7.8
$Strain_{AD}$ (%)	29.0 ± 6.5	27.9 ± 6.8	31.5 ± 8.8	28.6 ± 7.2	30.3 ± 6.8
IMT (mm)	0.34 ± 0.04	0.34 ± 0.06	0.37 ± 0.02	0.36 ± 0.05	0.37 ± 0.05
DC_{LD} ($mmHg^{-1} \times 10^{-3}$)	12.2 ± 4.3	12.3 ± 2.9	13.6 ± 4.9	12.1 ± 3.5	12.3 ± 3.1
DC_{AD} ($mmHg^{-1} \times 10^{-3}$)	10.4 ± 3.5	10.2 ± 2.8	11.6 ± 4.0	10.2 ± 3.2	10.3 ± 2.7

Values presented as mean ± standard deviation. One-way ANOVA. Pre=pre-pubertal; Early=early-pubertal; Peri=peri-pubertal; Late=late-pubertal; Post=post-pubertal; LD=lumen diameter; AD=arterial diameter, IMT=intima media thickness; CSA=cross-sectional area; DC=distensibility coefficient.

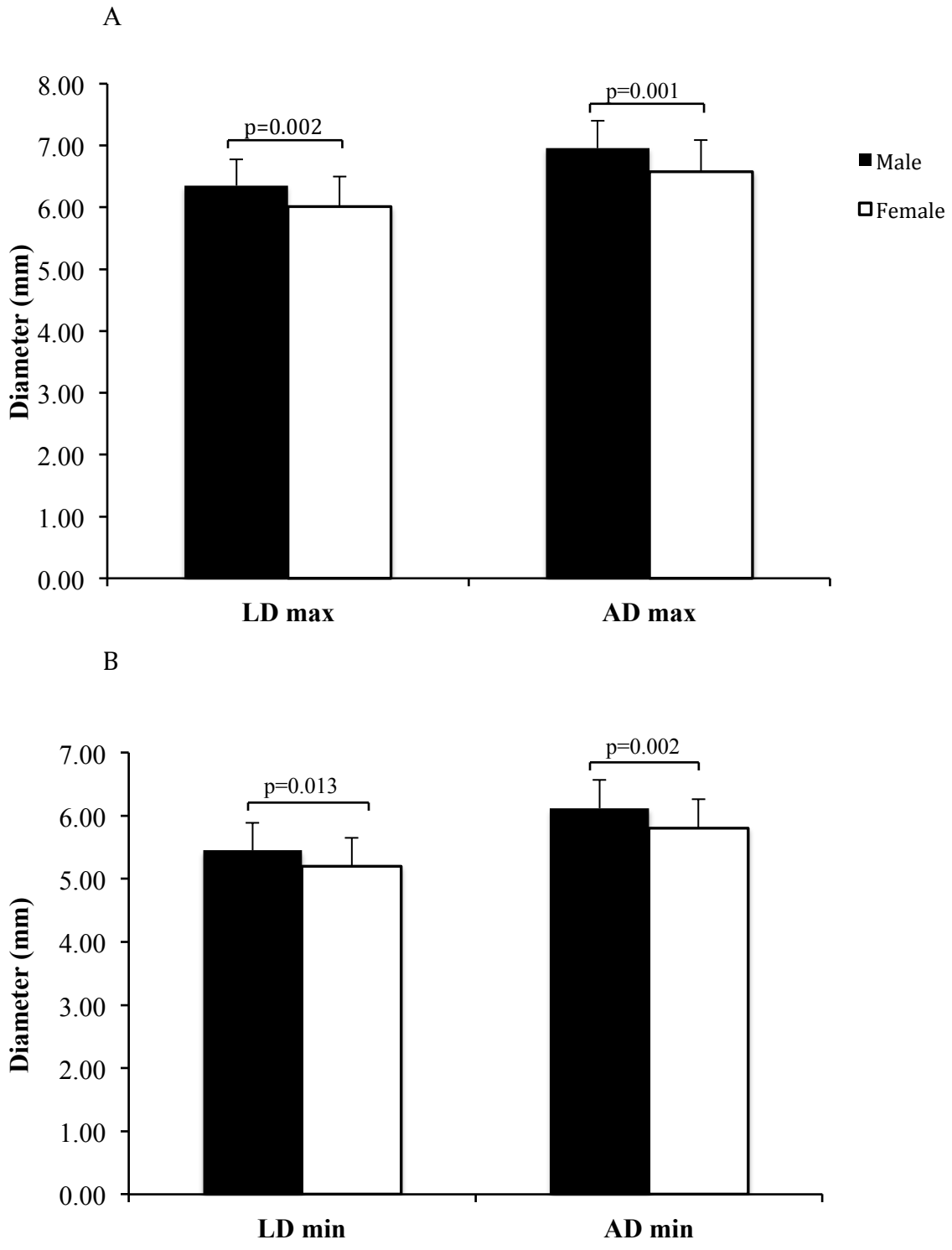

Figure 5-1
(A) Sex differences in maximum lumen and arterial diameters. (B) Sex differences in minimum lumen and arterial diameters.
Note: values presented as means and standard deviation for independent samples t-test.

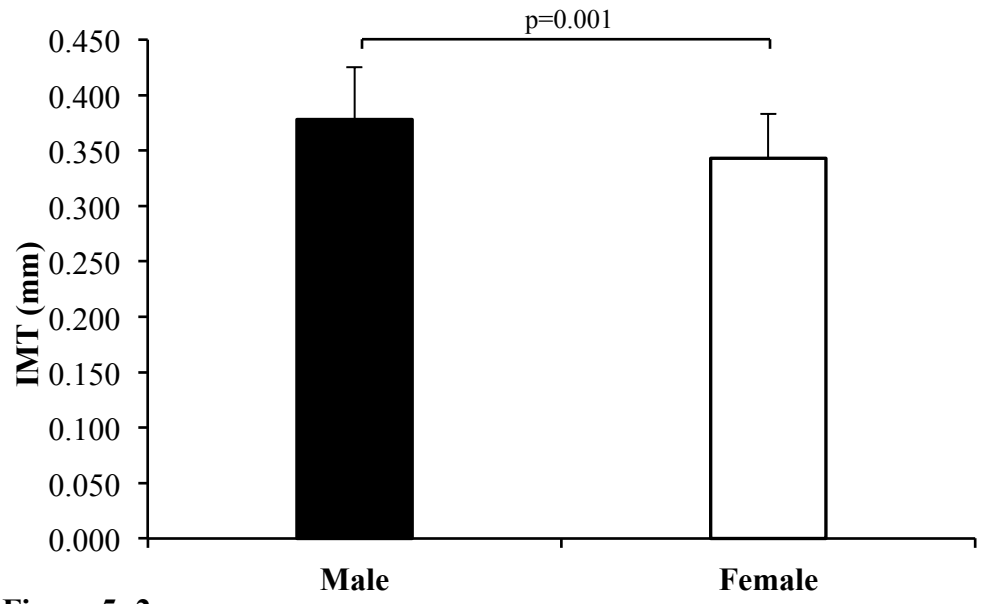

Figure 5- 2
Sex differences in intima-media thickness (IMT). Note: values presented as means and standard deviation for independent samples t-test.

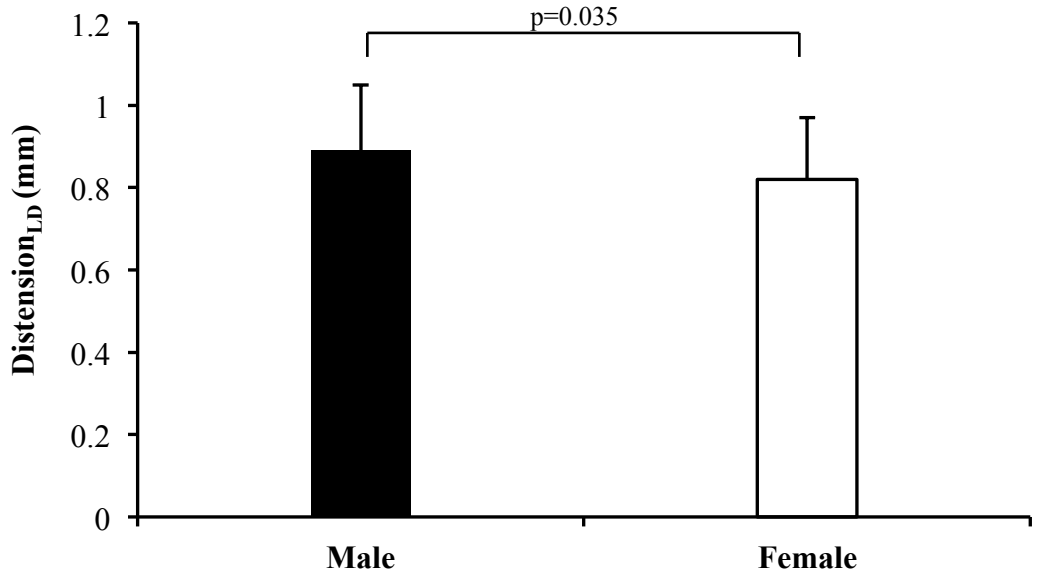

Figure 5-3
Sex differences in lumen diameter Distension (Distension$_{LD}$). Note: values presented as means and standard deviation for independent samples t-test.

Factors associated with arterial properties

Pearson's correlation analyses were completed for the total sample and separately for males and females to identify physiological factors (age, height, body mass, BMI, SBP, DBP, and RRI) associated with DC_{LD} (Table 5-3), DC_{AD} (Table 5-4), and IMT (Table 5-5). In the total sample DC_{LD} was correlated with DBP (r=-0.237, p=0.034) and MAP (r=-0.237, p=0.034), DC_{AD} was correlated with BMI (r=0.230, p=0.040), DBP (r=-0.263, p=0.018), and MAP (r=-0.252, p=0.024), and IMT was correlated with DBP (r=0.236, p=0.035) and MAP (r=0.253, p=0.024). In males, only IMT and BMI were correlated (r=0.348, p=0.032). In females, IMT correlated with DBP (r=0.382, p=0.012) and MAP (r=0.374, p=0.015), DC_{LD} was correlated with BMI (r=0.328, p=0.034), DBP (r=-0.322, p=0.028) and MAP (-0.274, p=0.046), DC_{AD} was correlated to body mass (r=0.340, p=0.027), BMI (r=0.405, p=0.008), and DBP (r=-0.322, p=0.038).

Table 5-3. Pearson's correlation of DC_{LD} with demographic, anthropometric and cardiovascular variables

	Total sample (n=80)		Males (n=38)		Females (n=42)	
DC_{LD}	**r**	**p**	**r**	**p**	**r**	**p**
Age (years)	-0.183	0.104	-0.217	0.190	-0.129	0.416
Height (cm)	-0.093	0.411	-0.195	0.240	0.052	0.745
Body Mass (kg)	0.049	0.669	-0.126	0.451	0.260	0.097
BMI (kg/m^2)	0.166	0.141	-0.015	0.930	**0.328**	**0.034**
SBP (mmHg)	-0.100	0.378	-0.081	0.629	-0.125	0.429
DBP (mmHg)	**-0.237**	**0.034**	-0.104	0.535	**-0.322**	**0.028**
MAP (mmHg)	**-0.237**	**0.034**	-0.139	0.405	**-0.274**	**0.046**
RRI (ms)	0.012	0.915	-0.015	0.930	-0.026	0.910

DC_{LD}=distensibility coefficient for lumen diameter; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; RRI=R-R interval.

Table 5-4. Pearson's correlation of DC_{AD} with demographic, anthropometric and cardiovascular variables

	Total sample (n=80)		Males (n=38)		Females (n=42)	
DC_{AD}	**r**	**p**	**r**	**p**	**r**	**p**
Age (years)	-0.145	0.199	-0.208	0.210	-0.077	0.628
Height (cm)	-0.072	0.523	-0.189	0.256	0.097	0.542
Body Mass (kg)	0.098	0.388	-0.121	0.470	**0.340**	**0.027**
BMI (kg/m^2)	**0.230**	**0.040**	-0.010	0.950	**0.405**	**0.008**
SBP (mmHg)	-0.090	0.429	-0.107	0.522	-0.071	0.656
DBP (mmHg)	**-0.263**	**0.018**	-0.196	0.238	**-0.322**	**0.038**
MAP (mmHg)	**-0.252**	**0.024**	-0.232	0.161	-0.274	0.079
RRI (ms)	0.001	0.994	0.019	0.911	-0.026	0.870

DC_{AD}=distensibility coefficient for arterial diameter; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; RRI=R-R interval.

Table 5-5. Pearson's correlation of IMT with demographic, anthropometric and cardiovascular variables

	Total sample (n=80)		**Males (n=38)**		**Females (n=42)**	
IMT	**r**	**p**	**r**	**p**	**r**	**p**
Age (years)	0.067	0.557	0.143	0.393	0.097	0.539
Height (cm)	0.165	0.142	0.143	0.391	0.158	0.317
Body Mass (kg)	0.104	0.360	0.264	0.110	-0.040	0.803
BMI (kg/m^2)	-0.015	0.893	**0.348**	**0.032**	-0.141	0.373
SBP (mmHg)	0.132	0.244	0.082	0.623	0.199	0.207
DBP (mmHg)	**0.236**	**0.035**	0.261	0.114	**0.382**	**0.012**
MAP (mmHg)	**0.253**	**0.024**	0.268	0.103	**0.374**	**0.015**
RRI (ms)	0.207	0.065	0.181	0.277	-0.043	0.786

IMT=intima-media thickness; BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; RRI=R-R interval.

Effect of Sex and Maturation on Arterial Properties

Two-way ANOVAs were completed for DC_{LD}, DC_{AD}, and IMT to assess the effects of maturation group and sex. An interaction term for maturation and sex was also included in the model. For DC_{LD} no main effect for sex ($F_{(1, 70)}$=0.462, ns), or maturation ($F_{(4, 70)}$=0.402, ns) were found, and no sex-by-maturation interaction ($F_{(4, 70)}$=4.729, df=4, ns) was found. Similarly for DC_{AD}, there was no main effect for maturation group ($F_{(4, 70)}$=0.573, ns), sex ($F_{(1, 70)}$=0.082, ns) or sex-by-maturation ($F_{(4, 70)}$=1.321, ns). For IMT, there was a main effect for sex ($F_{(1, 70)}$=11.465, p=0.001), but not for maturation ($F_{(4, 70)}$=1.545, ns) or their interaction ($F_{(4, 70)}$=0.763, ns). The sex effect remained after controlling for BMI ($F_{(1, 69)}$=0.433, ns), DBP ($F_{(1, 69)}$=9.196, p=0.003), and MAP ($F_{(1, 69)}$=5.616, p=0.021) separately.

Arterial Distensibility, IMT, and BRS

BRS for the 72 participants was 21.9 ± 9.2 ms/mmHg and ranged from 3.5-42.4 ms/mmHg. BRS was regressed on DC_{AD}, DC_{LD}, and IMT separately and included two models, which are presented in Table 5-6. DC_{AD}, DC_{LD}, and IMT were not significant predictors of BRS. This was also true after including sex and maturation into the model. Maturation was a significant predictor of BRS in all models (Table 5-6).

Table 5-6. Regression analyses of BRS on DC_{AD}, DC_{LD}, and IMT

BRS	**Model 1**	**p-value**	**Model 2**	**p-value**
DC_{AD}	0.322 (.114)	0.339	0.304 (0.108)	0.354
Maturation	-	-	-1.841 (-0.277)	**0.020**
Sex	-	-	0.122 (0.007)	0.954
Constant	18.476		26.644	
Adjusted R-squared	-0.001		0.050	
R^2	0.013		0.090	
DC_{LD}	0.351 (0.146)	0.223	0.344(0.143)	0.221
Maturation	-	-	-1.840 (-0.277)	**0.019**
Sex	-	-	0.273 (0.007)	0.898
Constant	17.459		23.449	
Adjusted R^2	0.007		0.059	
R^2	0.021		0.099	
IMT	-10.455 (0.054)	0.653	2.567 (0.013)	0.918
Maturation	-	-	-1.875 (-0.282)	**0.021**
Sex	-	-	0.212 (0.012)	0.926
Constant	25.692		27.028	
Adjusted R^2	-0.011		0.038	
R^2	0.003		0.079	

Values presented as b-coefficients with Beta in parentheses. BRS=baroreflex sensitivity; DC_{AD}=distensibility coefficient of arterial diameter; DC_{LD}=distensibility coefficient of lumen diameter; IMT=intima-media thickness.

Discussion

This novel study examined the effect of maturation and sex on CCA distensibility in children and adolescents, and its relationship to BRS. Overall, the findings suggest that CCA distensibility remains stable throughout maturation and is not a predictor of BRS. Furthermore, there was no evidence of a maturation effect on CCA properties; however, sex differences were evident with males having greater max and min diameters, IMT, and $Distension_{LD}$.

Maturation, Sex, and CCA Properties

Descriptive statistics (Table 5-2) demonstrated no maturational effect on arterial size or distensibility. There were distinct differences between males and females with respect to arterial size and structure. Males had greater IMT, maximum and minimum lumen and arterial diameters, and $Distension_{LD}$. Our findings are consistent with Sass and colleagues who found that CCA diameter did not change with age in 10-18 year olds, but a sex difference was evident after 14 years of age.[28] Likewise, CCA IMT did not increase with age. In contrast to the present findings, Sass and colleagues only found a sex difference after 18 years of age in which males had greater IMT than females.[28] Several studies have found IMT to increase slightly with age in children and adolescents[14, 29] with a sex difference appearing at 15 years.[14] In the present study IMT correlated with MAP and DBP in the total sample, with BMI in males, and DBP and MAP in females. Taken together, while it appears as though CCA diameter remains stable and is unaffected by growth and maturation in children and adolescents, changes in IMT with age are associated with body size and BP.[14, 28, 29]

CCA distensibility also remained stable throughout maturation. Additionally,

there was no sex difference in CCA distensibility. These findings correspond with previous studies in children and adolescents that suggest CCA distensibility is unaffected by sex and maturation.[15, 30] This is in contrast to other reports that have found sex specific changes in CCA distensibility and central pulse wave velocity with age and maturation.[10, 13, 14, 29] Ahimastos et al., (2003)[13] evaluated arterial stiffness by means of central pulse wave velocity by comparing pre- and post-pubertal males and females. Although females had stiffer central arteries compared to males pre-puberty, central stiffness decreased post-puberty in females and increased in males, eliminating sex differences. Similarly, Ayer et al., (2010)[31] found that augmentation index was greater in pre-pubertal females compared to males, independent of height and heart rate, indicating greater arterial stiffness in females. Collectively, these findings provide indirect support for the beneficial effect of estrogen on arterial stiffness and the notion that females possibly have intrinsically stiffer arteries in the absence of estrogen. Indeed, this is supported by a sex and age interaction in adults where CCA distensibility decreased more rapidly with age in women.[32, 33] Moreover, acute alterations in hormone concentrations during the menstrual cycle reveal that changes in circulating estrogen can alter CCA distensibility.[34] These findings are not universal as studies in children and adolescents, as well as adults, have failed to consistently find a sex difference in CCA distensibility and stiffness.[10, 30, 35, 36]

Arterial Distensibility and BRS

The cardiovagal baroreflex functions to maintain BP homeostasis on a continuous basis by reflexively adjusting RRI. Two important components of baroreflex function have been identified; the arterial mechanical component which is responsible for baroreceptor activation, and the neural component which incorporates afferent signaling

and central processing responsible for initiating changes in RRI.[1] The arterial mechanical component relies on the ability of the artery to respond to changes in pressure. Hence, arterial structure and function play a critical role in baroreflex function. The importance of CCA distensibility and thickness on BRS has been examined extensively in adults, with conflicting results. While several studies maintain that altered BRS is a result of changes in arterial properties,[4, 5, 7, 8, 37, 38] others suggest the neural component plays a larger role.[6, 9, 39] Taken together, these previous findings suggest that both components play a varying role in regulating BRS. Few studies exist in children and adolescents examining the relationship between CCA distensibility and BRS. Lenard and colleagues found that while BRS improved with age in children and adolescents, CCA distensibility decreased.[10] Therefore, improvements in BRS were attributed to neural improvements.[10] In the present study, CCA distensibility did not change with sex or maturation and was not a predictor of BRS. Therefore, it is likely that the decrease in BRS in males with maturation (Chapter 4) is a result of impaired neural function.

The neural component of BRS is comprised of afferent neural signaling, central processing, an efferent response, and cardiac responsiveness.[6] Central processing, acetylcholine production, and muscarinic receptor density are possible explanations to neural alterations with maturation. Sex hormones, particularly estrogen, elicit both genomic and non-genomic effects that can enhance BRS centrally,[40-43] increase acetylcholine concentration,[44] and increase muscarinic cell receptor density.[45] Less is known about testosterone; however, it has been shown to increase choline acetyltransferase activity,[46] as well as enhance BRS centrally.[47] Although direct conclusions cannot be drawn from this study and the mechanism of reduced BRS with

maturation in males and preserved BRS in females remains to be explained, it is possible that decreased BRS in males with maturation (Chapter 4) is due to the combination of increased SBP with maturation and lower sex hormone concentration due to the delayed onset of puberty in males, compared to females. It is also possible that throughout maturation females begin to rely more on changes in RRI, while males begin to rely more on changes in total peripheral resistance to regulate BP, as has been reported in adults.[48-50]

Although neural mechanisms provide a compelling explanation, it is plausible that methodological limitations prevented the ability to detect a relationship between CCA distensibility and BRS. Baroreceptors have been localized to the medial portion of the carotid sinus, distal to the carotid bifurcation.[51] Interestingly, digital subtraction angiography has demonstrated that the carotid sinus undergoes structural alterations in size in children and adolescents 10-19 years old compared to children 0-9 years old.[52] This suggests that maturation of the carotid sinus occurs, which in turn may influence maturational changes in BRS. Furthermore, it has been shown that changes in strain and distensibility occur earlier in the carotid sinus compared to the CCA.[53] More importantly, strain and distensibility of the proximal portion of the carotid sinus are reduced compared to other segments of the sinus.[52] Taken together, the aforementioned findings demonstrate that a possible explanation to the lack of relationship between BRS and arterial distensibility may be a result of using the CCA as a surrogate for the carotid sinus. Future work in children and adolescents necessitate the exploration of the role of carotid sinus distensibility on BRS to provide a more accurate assessment of the arterial mechanical component.

Limitations: The current study used self-reported Tanner stage to delineate maturation stage. Although this method has shown good agreement with physician reported maturation stage using Tanner criteria,[18] there may be bias in the self-reported stages, and difficulty in deciphering between stages. Furthermore, the timing and tempo of maturation was not evaluated because Tanner stage was reported on one visit. Therefore, a given pubertal group can encompass individuals at the early or late end of a given stage. Moreover, hormone concentrations were not collected in this study. Given the dynamic nature of pubertal development, no criteria are established linking a hormone concentration to a given pubertal stage.

Menstrual cycle phase was not controlled for in the present study. The influence of menstrual cycle phase on BRS and CCA distensibility has demonstrated inconsistent findings in adults.[34, 53, 54] To our knowledge, the influence of menstrual cycle phase on BRS and CCA distensibility has not been evaluated in adolescents. Given the greater menstrual cycle irregularity in adolescent girls and longer intermenstrual cycle length, hormone concentrations are likely to be lower and demonstrate more gradual variations compared to adults. Also, menstrual cycle irregularity increases the complexity of participant scheduling with no previous experimental evidence supporting its importance in adolescents. Therefore, not controlling for menstrual cycle phase in the present study is deemed to be acceptable and not believed to have impacted the results.

The sample size in the present study is small compared to previous studies examining maturation and CCA distensibility[15] and BRS.[10, 11] However, the lack of maturation group differences in CCA distensibility of the present study is similar to that of Marlatt et al., (2013)[15]. We observed small effect sizes for sex (0.14), maturation

(0.15), and their interaction (0.24) with respect to CCA distensibility. Based on these effect sizes the required sample size to obtain 80% power for sex would be 850, for maturation the required sample size would be 562, and for their interaction 207. In fact, the sample size in the aforementioned study by Marlatt and colleagues was 344. The need for such a high sample size illustrates the small main effects of sex, maturation, and their interaction on CCA distensibility. Therefore, although our sample size was small and power was low compared to previous studies, we observed similar effects in comparison to larger-scale studies. This implies that maturation has little effect on CCA properties. Lastly, it has been suggested that the measurement of arterial properties and BRS simultaneously on a beat-by-beat basis may better reflect the relationship between arterial mechanics and BRS.[1, 56] This method has been used in a variety of ways in adults, but not in children and adolescents.

In conclusion, the findings of the present study indicate that CCA distensibility is unaffected by maturation and is unrelated to BRS in children and adolescents. Furthermore, CCA diameter and IMT remain stable throughout maturation. Sex differences in CCA structure were evident, with males having greater diameters and IMT.

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Chapter 6: Study 3

Comparing peripheral pulse pressure to carotid sinus and common carotid pulse pressure in response to orthostatic stress in children and adolescents

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Abstract

Background: Carotid artery distensibility is an important predictor of baroreflex sensitivity at rest and in response to postural stress. Pulse pressure (PP) is an important stimulus for baroreceptor activation and local measures of arterial distensibility. The purpose of this study was to evaluate PP measured non-invasively at the common carotid artery (CCA), carotid sinus (CS), and finger; and to evaluate the change in PP at each location in response to a change in posture.

Methods: PP was measured at the CCA (PP_{CCA}) and CS (PP_{CS}) using applanation tonometry (Millar), and at the finger (PP_{Finger}) using photoplethysmography (Nexfin). PP was measured in supine and in a seated-recumbent position (SR) to elicit a postural stimulus. There were a total of 81 children and adolescents with PP measured at all three locations in supine, whereas 60 had all three measures in both supine and SR.

Results: PP_{CS} was greater than PP_{CCA} (34 ± 9 mmHg vs. 29 ± 8 mmHg, $p<0.001$), while PP_{Finger} was greater than both PP_{CCA} (46 ± 12 mmHg vs. 29 ± 8 mmHg, $p<0.001$) and PP_{CS} (34 ± 9 mmHg, $p<0.001$). PP_{Finger} significantly decreased from supine to SR ($p<0.001$), while there was no change in PP_{CCA} or PP_{CS} in response to posture. The relative change in PP_{Finger} was significantly different compared to the relative change in PP_{CCA} ($p<0.001$), and PP_{CS} ($p<0.001$). The relative change between PP_{CCA} and PP_{CS} were not different (8.7 ± 0.3% vs. 1.7 ± 0.3%, ns).

Conclusion: These findings highlight the importance of specificity of measurement site for PP and indicate PP measured at the finger does not accurately reflect PP at the CCA and CS in supine or in response to upright posture in children and adolescents. Furthermore, these findings indicate that PP_{CCA} and PP_{Finger} may not be suitable surrogates of PP_{CS}. This is important to consider when evaluating the impact of arterial mechanical properties on BRS.

Introduction

Central aortic pressure and stiffness have been shown to be important predictors of cardiovascular events and mortality.[1, 2] Recently, reduced common carotid artery (CCA) distensibility has also been shown to be an important predictor of cardiovascular events and all-cause mortality.[3] Determining CCA distensibility relies on accurate measurement of local pulse pressure (PP), typically using applanation tonometry at the carotid artery.[4-6] PP is a major determinant of large artery remodeling[7, 8] and has been identified as an important independent risk factor for cardiovascular events.[1, 9] Furthermore, PP is an important stimulus for arterial baroreceptor activation.[10, 11] Therefore, PP is an essential component for both arterial distensibility and baroreflex activation.

Baroreceptors respond to deviations in blood pressure (BP) by reflexively altering heart rate (HR). This can be determined by relating the change in R-R interval (RRI) for a given change in systolic BP (SBP), which provides a measure of cardiovagal baroreflex sensitivity (BRS). The importance of CCA distensibility on BRS has been demonstrated previously.[12-14] Furthermore, the baroreflex can be delineated into two components; the mechanical and neural components.[15] The mechanical component refers to the mechanical transduction of BP into carotid stretch and the neural component refers to the neural transduction of carotid stretch into cardiac vagal outflow. The mechanical component represents the vascular properties of the artery, such as distensibility, and is responsible for baroreceptor activation.[15] It is quantified as the change in CCA diameter for a given change in SBP typically measured peripherally at the finger.

The accurate representation of local PP is important when examining BRS and the impact of mechanical properties on BRS.[6, 16] This is especially true in scenarios such as orthostatic stress, where gravitational stressors may alter local pressures, as well as HR.[17] Indeed, it has been shown previously that change in peripheral PP (finger) does not reflect CCA PP in response to head-up tilt (HUT) in adults.[18] However, no study has examined PP at the carotid sinus (CS) in comparison to the CCA or to peripheral measures of PP. This is important to consider as baroreceptors are localized to the CS.[19, 20] Likewise, the use of peripheral pressure measurements for the assessment of central pressures has been questioned[6, 16] based on the principle of pressure amplification in peripheral arteries.[21, 22] This may be particularly important to address in children and adolescents as differences in central and peripheral pressures are more pronounced and aging is associated with increasing central arterial stiffness.[22-24] Despite these findings, and caution raised by others,[6, 18, 25] the use of peripheral PP to assess the impact of mechanical properties of the CCA on BRS in response to orthostatic stress is still common.[26, 27]

Therefore, the purpose of this study was two-fold, 1) to evaluate the differences in PP measured at the finger (PP_{Finger}), CCA (PP_{CCA}) and CS (PP_{CS}) in supine, and 2) to evaluate the differences in response to a moderate postural stimulus at each measurement site.

Methods

Study Participants

The present study included 81 of the 121 participants from Chapter 5 who had PP measurements at three recording sites (finger, CCA, CS) in supine. Of the 81 participants,

60 had PP measurements at all three sites for both the supine and seated recumbent (SR) positions. All of the participants were between 8-18 years of age and were healthy with no prior history of chronic illness. They were asked to fast four-hours prior, avoid vigorous physical activity, smoking, and caffeine 12-hours prior to data collection. The Brock University Research Ethics Board approved this study.

Experimental Protocol

Upon arrival to the laboratory, the parent/guardian(s) of the participants provided informed written consent if the participant was underage, and the participant provided assent. Information was collected on medical conditions and/or medications. Following this, height and body mass were collected. Participants then began cardiovascular testing. First, they were asked to lie supine for a period of 15 minutes to allow BP and HR to achieve baseline levels. Four initial manual BPs were measured and the last three were averaged. Beat-by-beat data collection of HR and BP began and continued for at least five-minutes while the subject continued to rest in a dim, quiet, and temperature controlled setting. This five-minute recording of BP collection of the left middle finger was used for the measurement of PP_{Finger}. As well, measurements of PP_{CCA} and PP_{CS} were obtained using applanation tonometry (Millar). Participants were then passively seated in a 50° recumbent (SR) upright position with their back supported and legs extended, while their finger remained at heart level. After a five-minute stabilization period, all measurements completed in the supine position were repeated.

Experimental measures

Anthropometry

All measurements of body composition were performed in a private room with the option of having the parent/guardian(s) present. Body mass index (BMI) was calculated using height and body mass (kg/m^2). Standing height (cm) was measured using a stadiometer (Stat 7X, Ellard Instrumentation 50 Ltd Monroe, WA, USA) with the participants' shoes removed and recorded to the nearest 0.1 cm. Body mass (kg) was measured using a digital scale (BWB-800S, Tanita Digital Scale, Tokyo, Japan) and recorded to the nearest 0.1 kg.

Blood Pressure

Auscultation is the recommended method of BP measurement in children.[28] Participants lied supine with their arm resting at heart level for 15 minutes. In both the supine and SR positions, BP was measured four times using a non-invasive, standard inflatable cuff and a sphygmomanometer placed on the right arm. The last three were recorded and averaged to determine SBP, diastolic BP (DBP), and mean arterial pressure (MAP). A stethoscope was placed over the brachial artery pulse, below the bottom edge of the cuff (2 cm above the cubital fossa). Correct measurement of BP requires an appropriate sized cuff; each child was fitted with either a pediatric or adult cuff based on arm size.[28] MAP was calculated using the formula $MAP = 1/3 \cdot SBP + 2/3 \cdot DBP$.

Beat-by-beat HR and BP

Following 15 minutes of supine rest, five minutes of beat-by-beat HR and BP data were collected simultaneously. Beat-by-beat BP (SBP and DBP) was collected using photoplethysmography (Nexfin, BMEYE, Amsterdam, The Netherlands) from the middle

finger of the left hand. Since BP taken at the finger slightly differs from that taken at the arm, the average manual BPs taken at the beginning of the beat-by-beat recording were used to adjust the beat-by-beat values collected simultaneously with photoplethysmography. HR was collected using a standard single-lead electrocardiogram. The average of the five-minute recording was used to determine PP_{Finger}, which was calculated as the difference between SBP and DBP.

Arterial Tonometry

Applanation tonometry was used to non-invasively determine left PP_{CCA} and PP_{CS}. A hand-held tonometer (Millar Instruments, Houston Texas) was used to manually obtain pressure waveforms from the left CCA and CS. In order to ensure accurate measurement of PP_{CS}, the CS was landmarked using ultrasound sonography. The transducer was calibrated with an external device utilizing a two-point calibration system. The pressure wave obtained is very similar to that recorded within the artery.[22, 29] Since the pressure required to applanate the artery and compress overlying structures varies, the absolute values of systolic and diastolic pressure are not reliable, but the amplitude (PP) can be determined reliably.[18] Accuracy of the Millar devices at the sites of the carotid and radial arteries has been established.[22, 29] In order to obtain a suitable waveform the criteria outlined by Chen et al., (1996)[29] were followed so that the operator adjusted the hold-down pressure so that acceptable waveforms had a stable baseline, maximum amplitude, and a reasonable configuration. Pressure measurements from 10-15 cardiac cycles were used to determine PP_{CCA} and PP_{CS} as the difference between the maximum and minimum pressure values. An example of pressure waveforms obtained for the CCA (Appendix A-2) and CS (Appendix B-2) are provided.

Statistical Analysis

Statistical analyses were performed using IBM Statistics (IBM SPSS Statistics 20). Continuous variables are presented as mean ± standard deviation (SD) and all categorical variables are presented as proportions (%). All assumptions were tested prior to completing parametric tests. Normality was tested using the Shapiro-Wilk test and sphericity was tested by the Mauchly's test. Box plots were used to assess outliers in the data.

A Chi-Square test was used to examine proportions of males and females. Pearson's correlation and linear regression analyses were completed between measurement sites in the supine position. A repeated-measures ANOVA was performed to assess the effect of measurement site on supine PP. A Bonferroni post-hoc test was used to identify significant differences in supine PP between measurement sites. Paired t-tests were used to examine the effect of posture on PP at each measurement site. A repeated-measures ANOVA was completed to examine the effects of measurement site (i.e. CCA, CS, and finger) and posture (i.e. supine, SR) on relative change in PP, while controlling for baseline differences. A Bonferroni post-hoc test was used to determine significant differences between measurement sites. Significance was set at $p<0.05$ (two tails).

Supine PPs at each site were not normally distributed. There was no evidence of severe skewness or kurtosis in any of the measurement sites. Sphericity was not assumed ($W=0.907$, $p=0.02$) for the supine sample, and therefore, the Greenhouse-Geisser correction was used for repeated-measures ANOVA. For the sample with all three measurements in both the supine and SR positions, only relative change in PP_{CCA} was

normally distributed. There was no severe skewness or kurtosis for relative change at any measurement site, and Sphericity was assumed. The sample was deemed large enough to proceed with parametric testing. Outliers were included in the analysis as results did not differ when excluded.

Results

Baseline Characteristics

Table 6-1 presents participant demographic and anthropometric characteristics for the 81 participants. There was an equal distribution of males and females in the sample ($\chi^2_{(1, 81)}$=0.012, p=0.912). The average age of the sample was 13.5 ± 2.7 years, with an average height and body mass of 158.3 ± 13.9 cm and 50.7 ± 15.0 kg, respectively.

Effect of Measurement Site on Supine PP

A measurement site effect ($F_{(1.83, 146.35)}$ = 96.056, p<0.001) on PP was found using a repeated-measures ANOVA. Figure 6-1 graphically depicts the results of the between measurement site comparisons. Bonferroni post-hoc analysis identified differences between each site in the supine position. PP_{CS} was greater than PP_{CCA} (34 ± 9 mmHg vs. 29 ± 8 mmHg, p<0.001), while PP_{Finger} was greater than both PP_{CCA} (46 ± 12 mmHg vs. 29 ± 8 mmHg, p<0.001) and PP_{CS} (34 ± 9 mmHg, p<0.001). Table 6-2 displays correlations of PP between measurement sites in the supine position. All measurements were significantly correlated. PP_{CS} correlated best with PP_{Finger}.

Table 6-1: Participant characteristics

	Mean ± SD
n	81
Male (%)	49.4
Age (years)	13.5 ± 2.7
Height (cm)	158.3 ± 13.9
Body Mass (kg)	50.7 ± 15.0
BMI (kg/m^2)	19.8 ± 3.5
HR (bpm)	66 ± 10
SBP (mmHg)	110 ± 10
DBP (mmHg)	63 ± 7
MAP (mmHg)	78 ± 6

BMI=body mass index; HR=heart rate; bpm=beats per minute; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure.

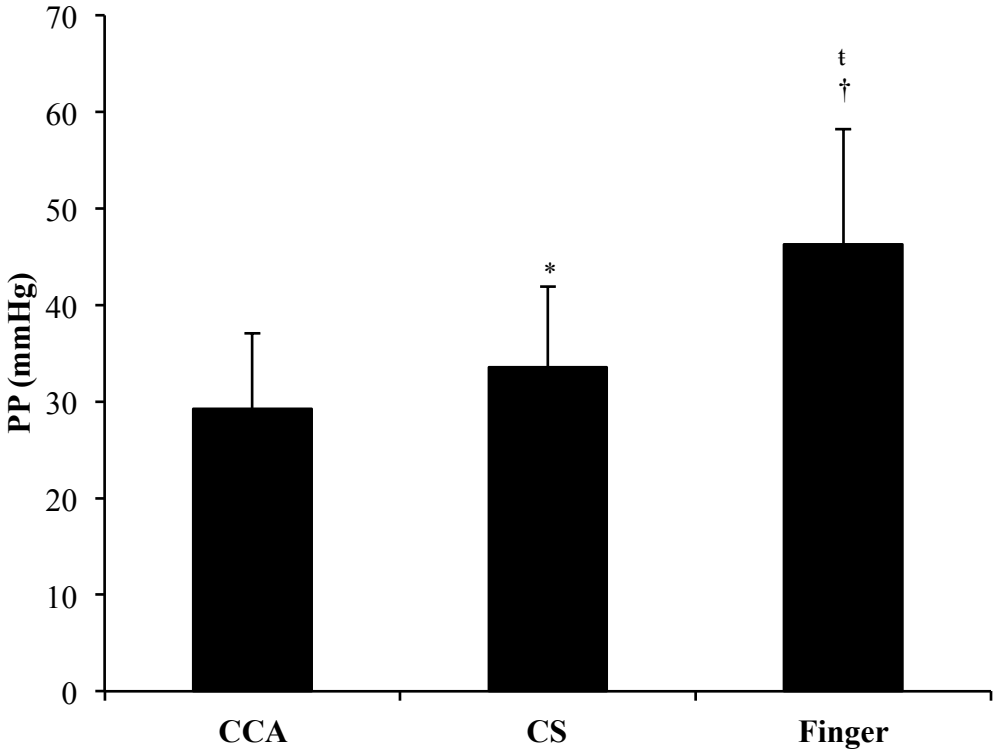

Figure 6-1
Comparison of pulse pressure (PP) measured at the common carotid artery (CCA), carotid sinus (CS) and finger in the supine position. Note: *significantly different from CCA, $p<0.05$; ^{t}significantly different from CCA, $p<0.001$; †significantly different from CS, $p<0.001$.

Table 6-2: Linear regression and correlation coefficients of pulse pressure measured at the common carotid, carotid sinus and left middle finger in the supine position.

	r	B (SE)	R^2	p-value
CCA vs. Finger	0.227	0.150 (0.072)	0.052	0.041
CS vs. Finger	0.395	0.309 (0.081)	0.156	<0.001
CCA vs. CS	0.377	0.317 (0.088)	0.142	0.001

CCA=common carotid artery; CS=carotid sinus; B= b-coefficient; SE=standard error.

Effect of Posture and Measurement Site on PP

Table 6-3 presents the PP values at each measurement site for the 60 participants that had all three measurements in supine and SR positions. Paired t-tests (Table 6-3) revealed that PP_{Finger} significantly decreased from supine to SR (t=5.817, p<0.001), while there was no change in PP_{CCA} (t= -1.603, ns) or PP_{CS} (t=0.378, ns) in response to SR.

When comparing the relative change in PP (Figure 6-2), a repeated-measures ANOVA revealed a measurement site effect ($F_{(2, 120)}$=10.680, p<0.001). A Bonferroni post-hoc test identified that the change in PP_{Finger} was significantly different compared to the change in PP_{CCA} (-11.7 ± 0.2% vs. 8.7 ± 0.3%, p<0.001), and PP_{CS} (1.7 ± 0.3%, p=0.007). The change between PP_{CCA} and PP_{CS} was not different (8.7 ± 0.3% vs. 1.7 ± 0.3%, ns).

Table 6-3: Pulse pressure measured at the common carotid, carotid sinus and left middle finger in the supine and seated-recumbent positions (n=60).

	S	**SR**
PP_{CCA} (mmHg)	29 ± 8	31 ± 7
PP_{CS} (mmHg)	34 ± 8	33 ± 9
PP_{Finger} (mmHg)	47 ± 12	40 ± 10*

Values presented as mean ± SD. S=supine; SR=seated-recumbent. PP_{CCA} =common carotid artery pulse pressure; PP_{CS}=carotid sinus pulse pressure; PP_{Finger}=finger pulse pressure. Note: Paired t-test *significantly different compared to S, $p<0.001$.

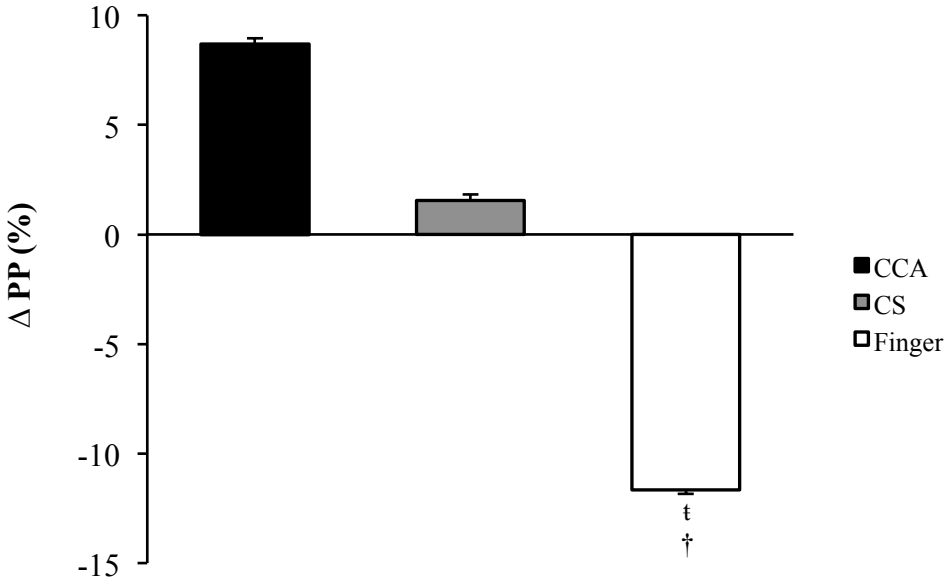

Figure 6-2
Relative change in pulse pressure (PP) in response to seated-recumbent position at the common carotid artery (CCA), carotid sinus (CS) and finger. Note: [t]significantly different from CCA $p<0.001$; [†]significantly different from CS $p<0.001$.

Discussion

The present study compared PP measured peripherally at the finger, using photoplethysmography, to that measured centrally at the CCA and CS, using applanation tonometry. The important findings from this study are two-fold; first, PP measured at the finger was greater than that of both the CCA and CS in supine. In addition, PP at the CS was greater than the CCA. Second, PP measured at the finger decreased in response to a moderate posture stimulus, while CCA and CS pressures were unchanged. These findings suggest that pressure measured at the finger does not represent pressure measured at the CCA and CS. This is important to consider when evaluating the effect of mechanical arterial properties on BRS.

Our findings are consistent with a previous study examining central and peripheral PP in response to HUT in adults.[18] However, we have extended these previous findings in two important ways; 1) these findings apply to children and adolescents and 2) PP measured at the CS is greater than the CCA in supine, and responds similarly to the CCA to a postural stimulus. Steinback et al., (2004)[18] found that peripheral PP measured at the radial artery and finger were greater than that of the CCA. Furthermore, peripheral PP decreased in response to tilt, while central PP remained unchanged. Our findings support these results and extend them further to the CS. This is important as baroreceptors are densely localized in the body of the CS, just distal to the bifurcation.[19] Our values slightly differ from those reported by Steinback.[18] In their study PP_{Finger} and PP_{CCA} were 55 ± 4 mmHg and 33 ± 5 mmHg, respectively, while ours were 46 ± 12 and 29 ± 8, respectively. Furthermore, PP_{CS} in our study was 34 ± 8 mmHg. When examining the change in PP at the different measurement sites, our findings are consistent with

Steinback and colleagues[18] and further suggest that the CS responds in a similar manner to the CCA. The magnitude of change at the peripheral site in the present study was lower compared to Steinback et al., (2004).[18] This may be due to the moderate postural stimulus used in the present study. We employed a postural stimulus that involved passively moving each participant into a seated-recumbent position at an angle of 50° with their back supported and legs extended, while Steinback used 60° HUT. Therefore, the difference in stimulus likely accounts for the magnitude difference between studies.

BRS is determined by relating the change in HR for a given change in pressure, whereby a greater change in HR for a given change in pressure is associated with a greater sensitivity and better autonomic regulation.[30] Two important components of BRS are the mechanical and neural components. The mechanical component relates the change in arterial distension (typically measured at the CCA) to a given change in SBP (typically measured at the finger).[15, 27] Distension is important for baroreceptor activation and reflects local arterial stiffness. The neural component can be determined by relating the change in HR for a given change in distension.[15, 27] Several groups have effectively quantified the individual components to examine their independent effects on BRS in response to orthostatic stress.[26, 27] However, these past studies relate SBP recorded from the finger to CCA distension, which may not reflect local pressure at the CCA. Consequently, this may not accurately reflect the impact of the arterial mechanical component on BRS. A study by Kornet et al., (2002)[31] highlighted this point by demonstrating that distension rate measured at the CCA is a better predictor of RRI variability than SBP measured peripherally.

Another important finding of the present study was that PP_{CS} was greater than

PP_{CCA} in the supine position (Figure 6-1) suggesting possible differences in elastic properties between the two arterial sites, although the relative change in response to SR posture did not differ between the CCA and CS (Figure 6-2). This may have important implications for the impact of arterial mechanical properties on BRS. As previously mentioned, baroreceptors are densely localized in the body of the CS, just distal to the bifurcation. As well, CS nerve connections have been shown to terminate at the level of the CS.[19, 20] This location appears to be more susceptible to atherosclerotic lesions and increases in stiffness compared to the CCA.[32-34] Due to the difficulty in obtaining ultrasound images of the CS, and the possibility of the ultrasound probe and tonometer causing baroreceptor activation, the CCA has been used as a surrogate for the CS.[12-15, 35] However, it may be useful to examine the mechanical properties of the CS when assessing the impact of arterial mechanical properties on BRS. This is of importance for children and adolescents as the CS has been shown to exhibit maturational changes, whereby the root of the CS increases in size during adolescence compared to the CCA.[36] Future research should explore the role of CS distensibility on BRS in comparison to CCA distensibility in children and adolescents.

The use of continuous finger arterial pressure recordings has been shown to be an accurate representation of intra-arterial brachial pressure.[37, 38] The peripheral arteries are subject to pressure amplification compared to central arteries[21, 22] and the difference increases with increased HR and MAP.[17] Pressure amplification in peripheral arteries explains the greater PP found in the CS compared to the CCA, and the finger compared to the CCA and CS. It is important to note that PP measured using hand-held tonometry (Millar) has demonstrated lower values compared to other non-invasive and invasive

methods measured at the same site.[18, 25] This may explain the differences detected between the finger and the CCA and CS in the present study. However, as previously suggested, this would not affect the relative change in PP seen in the present study in response to SR posture.[18] More importantly, this would not affect the difference detected between the CCA and CS in the supine position, as the same device (Millar) was used for both. Additionally, the ability of the Millar to track PP over a wide pressure range has been demonstrated.[18, 39] Therefore, the difference in PP responses between the peripheral and central arteries may have important implications for the evaluation of arterial properties.[6, 17]

Limitations: According to the theory of applanation tonometry, when the wall of an artery is flattened (applanated) by the probe, the contact pressure between the probe and the wall is equal to intra-arterial pressure.[29] This method is subject to hold down pressure which can provide inaccurate absolute BP values. Furthermore, pressure values obtained with this technique can be affected by the amount of neck tissue, as well as breathing artifact.[40] In an attempt to address these measurement issues, calibration techniques have been used to adjust tonometric values obtained at the CCA to brachial pressures.[25, 29, 41] This was not employed in the present study due to the fact that adjusted carotid pressures mirrored brachial pressures, implying no pressure amplification between the carotid and brachial arteries. O'Rourke and colleagues have recently questioned the use of this calibration technique.[42, 43]

Although there is no direct guide to indicate optimal applanation, it is believed that this occurs when the operator adjusts the hold-down force so that the waveform has a stable baseline, maximum amplitude, and a reasonable configuration.[29] This is sometimes

difficult to achieve and hence, a training period was implemented to obtain reasonable carotid waveforms. Furthermore, careful attention was given to accurately detect maximum and minimum pressures free of artifact.

In summary, the findings of the present study demonstrated differences in PP measured at the CCA, CS, and finger in children and adolescents. Furthermore, these measurement sites responded differently to a posture stimulus where PP_{Finger} decreased in response to SR posture, while PP_{CCA} and PP_{CS} did not change. These findings have important implications for the measurement of local arterial distensibility in supine and upright posture, and its impact on BRS in children and adolescents.

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Chapter 7: Study 4

Comparing carotid sinus and common carotid distensibility and their relationship to baroreflex sensitivity in children and adolescents in response to a moderate postural stimulus.

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Keywords: arterial distensibility, carotid sinus distensibility, common carotid artery, orthostatic stress, children, and adolescents.

Author Contributions:
Daniele Chirico: designed the study and conceived the idea, collected and analyzed the data, and wrote the manuscript

Dr. J. Kevin Shoemaker: gave input to the study design and helped conceive the idea, assisted with interpretation of results, and reviewed and critiqued the manuscript

Dr. Panagiota Klentrou: gave input to the study design, assisted with interpretation of results, and reviewed and critiqued the manuscript

Dr. Jian Liu: gave input to the study design, assisted with statistical analyses and interpretation of results, and reviewed and critiqued the manuscript

Dr. Deborah D. O'Leary: supervised the work, assisted with conceiving the idea, and reviewed and critiqued the manuscript

Abstract

Background: Several studies have examined the relationship between common carotid artery (CCA) distensibility and baroreflex sensitivity (BRS) in response to posture change in adults, but no such study exists in children and adolescents. Furthermore, no study has assessed the relationship between carotid sinus (CS) distensibility and BRS in response to a postural stimulus. Therefore, we tested the hypotheses that a change in BRS in response to posture change is associated with a change in arterial mechanical properties, and the CS is a better predictor of this change than the CCA.

Methods and Results: Twelve children and adolescents (4 males and 8 females) between 11.4-17.4 years of age were included in this study. Ultrasound sonography and applanation tonometry were used to determine the distensibility coefficient (DC) at the CCA (DC_{CCA}) and CS (DC_{CS}) in the supine and seat-recumbent (SR) positions. As well, beat-by-beat blood pressure (BP) and R-R interval (RRI) were collected in the supine and SR positions in order to determine BRS. BRS was assessed by transfer function analysis in the low frequency range (0.05–0.15Hz). The results demonstrated that DC_{CCA} and DC_{CS} were not different in supine and were significantly correlated ($r=0.618$, $p=0.032$). However, in response to SR there was a significant decrease in DC_{CS} (10.3 ± 2.9 vs. 8.1 ± 1.7 $mmHg^{-1} \times 10^{-3}$, $p=0.001$), but not DC_{CCA} (10.3 ± 2.7 vs. 9.5 ± 2.1 $mmHg^{-1} \times 10^{-3}$, ns). Furthermore, regression analyses revealed that the relative change in BRS in response to SR was significantly correlated to the relative change in DC_{CS} ($B=1.81$, $R^2=0.573$, $p=0.004$), but not DC_{CCA} ($B=-0.077$, $R^2=0.002$, $p=0.892$).

Conclusion: The findings of the present study indicate that the CCA and CS respond differently to posture change in children and adolescents, and the relative change in BRS is correlated with that of the DC_{CS}, not DC_{CCA}. Therefore, the CS appears to be more suitable to evaluate the effect of arterial distensibility on BRS in response to posture change.

Introduction

Arterial baroreceptors are stretch sensitive receptors that respond to mechanical deformation. Baroreceptors, which are located in the carotid sinus (CS) and aortic arch, regulate blood pressure (BP) on a beat-by-beat basis.[1] Baroreceptors respond to increases in pressure such that an increase in pressure causes an increase in baroreceptor firing. Changes in arterial baroreceptor afferent discharge are transmitted to the nucleus of the solitary tract (NTS), via the glossopharyngeal nerve.[2] This triggers reflex adjustments in R-R interval (RRI) and vascular resistance that buffer or oppose changes in BP: a rise in pressure elicits reflex parasympathetic activation and sympathetic inhibition resulting in a decrease in HR and vascular resistance.[1] Cardiovagal baroreflex refers to the reflex adjustments in RRI for a given change in systolic BP (BP).

Baroreceptor activity is linked to the mechanical properties of the tissues to which they are bound.[3] Landgren et al., (1952)[4] demonstrated a relationship between CS diameter and baroreceptor nerve activity. This data showed that baroreceptor firing is proportional to changes in CS diameter. Therefore, the ability of an artery to distend in response to increased pressure plays an important role in baroreceptor activity. As a result, arterial distensibility is an important determinant of the magnitude of baroreceptor deformation, and baroreflex function.[4, 5]

Cardiovagal baroreflex function relies on two important components, which can be delineated into a mechanical (arterial) and neural component.[6] The mechanical component represents the vascular properties of the artery and is responsible for baroreceptor activation. It is quantified as the change in diameter for a given change in pressure. The neural component, which is quantified by the change in RRI to a given

change in diameter, reflects the neural transduction of arterial stretch into vagal outflow.[6] Hence, integrated cardiovagal baroreflex sensitivity (BRS) is defined as a change in RRI for a given change in pressure (ms/mmHg).

Using a variety of methodologies, several investigators have evaluated the individual components and related them to changes in BRS.[6-12] Monahan found that common carotid artery (CCA) compliance was strongly correlated with age in healthy adults, and increased CCA compliance with exercise was associated with improved cardiovagal BRS.[9] Kornet et al., (2002)[13] further suggested that distension and relative distension were the best predictors of RRI and may be better for evaluating BRS. These findings are supported by others[11, 14, 15] and emphasize the importance of arterial distensibility on BRS. Conversely, others have suggested that the neural component plays a more important role in BRS alterations.[7, 10, 12] A study by Lenard et al., (2004)[16] extended the assessment of CCA distensibility and BRS to children and adolescents with respect to age and found that BRS increased with age, while CCA distensibility decreased. Additionally, we have demonstrated that alterations in BRS with maturation are not related to CCA distensibility in children and adolescents (Chapter 5). Taken together, these findings suggest that change in BRS with aging and maturation in children and adolescents is associated with neural alterations, which is consistent with aging adults.[7]

Diminished cardiovagal baroreflex function has been observed in response to orthostatic stimuli such as head-up tilt (HUT).[10, 11, 15, 17] The mechanism for decreased BRS is unknown; however, investigators have evaluated the mechanical and neural components of BRS in order to elucidate a mechanism. While some studies have

attributed reduced BRS to arterial changes of the CCA,[11, 15] others have attributed reduced BRS in response to orthostatic stress to reductions in the neural component.[10] Although BRS is diminished in response to postural stimuli in children and adolescents as well,[18] the relationship between arterial distensibility and BRS in response to a postural stimulus has not been evaluated.

The lack of relationship between arterial distensibility and BRS reported in previous studies may be a result examining arterial distensibility at the CCA, rather than the CS, where baroreceptors are densely localized.[19] Furthermore, it has been shown that central and peripheral pulse pressure (PP) vary in their responses to orthostatic stress,[20] (Chapter 6) and that CS PP is greater than CCA PP in children and adolescents (Chapter 6). Therefore, it may be advantageous to examine the relationship between CS distensibility and BRS in response to a postural stimulus. This is further supported by findings from Van Merode et al., (1993)[21] who showed that changes in CS distensibility occur prior to changes in CCA distensibility in borderline hypertensive, and aging adults.[21] This suggests heterogeneity in distensibility changes between the CS and CCA. Moreover, it has been shown that the proximal portion of the internal carotid artery (ICA), where baroreceptors are located, demonstrates significant growth during adolescence compared to the CCA.[22] Therefore, it is reasonable to believe that the response of the CS may differ compared to the CCA and may have a more influential role on BRS. Hence, the purpose of this study was to examine the relationship between CS and CCA distensibility and BRS in response to a postural stimulus in children and adolescents. It was hypothesized that a change in BRS in response to posture change is associated with a change in arterial mechanical properties, and that the CS is a better

predictor of this change than the CCA. A moderate posture stimulus was utilized by passively moving children from a supine to a seated-recumbent (SR) position with their legs extended and supported.

Methods

Sample Population

A subsample of 18 participants from Chapter 5 was tested for this study. Of those, 12 participants were able to complete the entire protocol in which adequate data collection occurred. Two participants were excluded due to medications, one additional participant could not complete the upright posture protocol, and the remaining three were lost due to inadequate carotid sinus image quality. In all, 4 boys and 8 girls were included in the final sample (mean age 14.4 ± 2.1 years). Participants were scheduled for one, two-hour appointment at the Human Hemodynamic Laboratory at Brock University. They were asked to fast four-hours prior to their appointment and to avoid vigorous physical activity, smoking, and caffeine 12-hours prior to their appointment. The Brock University Research Ethics Board approved this study.

Experimental Protocol

Upon arrival to the laboratory, the parent/guardian(s) of the participants provided informed written consent if the participant was underage, and the participant provided assent. Information was collected on medical conditions and/or medications. Following this, height and body mass were collected. Participants then began the cardiovascular assessment. First, they were asked to lie supine for a period of 15 minutes to allow BP and heart rate (HR) to reach baseline levels. Four initial manual BPs were measured and the last three were averaged. Beat-by-beat data collection began and continued for at least

five minutes while the subject continued to rest in a dim, quiet, and temperature controlled setting. Following the five-minute baseline collection, ultrasound sonography and tonometry were completed for the CCA and the CS. Participants were then passively seated in a recumbent (SR) upright position at an angle of 50° with their back supported and legs extended, while their finger remained at heart level. After a five-minute stabilization period, all measurements completed in the supine position were repeated.

Experimental measures

Anthropometry

All anthropometric measurements were performed in a private room with the option of having the parent/guardian(s) present. Body mass index (BMI) was calculated using height and body mass (kg/m^2). Standing height (cm) was measured using a stadiometer (Stat 7X, Ellard Instrumentation 50 Ltd Monroe, WA, USA) with the participants' shoes removed and recorded to the nearest 0.1 cm. Body mass (kg) was measured using a digital scale (BWB-800S, Tanita Digital Scale, Tokyo, Japan) and recorded to the nearest 0.1 kg.

Sexual Maturation

Secondary sex characteristics were assessed using the criteria of Tanner.[23, 24] To classify subjects into their maturation grouping, pubertal maturation was self-reported using pictures of the Sexual Maturation Scale by Tanner taken from Taylor et al (2001).[25] To reduce embarrassment, each subject completed the self-assessment in a private room with the option of having their parent(s) present. Pubertal maturation was categorized based on self-assed pubic hair as it has been shown to have better agreement with physical examination by a physician compared to breast/genitalia development.[25]

Participants were organized into their maturation groups based on the following: pre-pubertal (Pre, Tanner stage 1), early-pubertal (Early, Tanner stage 2), peri-pubertal (Peri, Tanner 3), late-pubertal (Late, Tanner 4), and post-pubertal (Post, Tanner 5 & 6).

Blood Pressure

Auscultation is the recommended method of BP measurement in children.[26] Participants lied supine with their arm resting at heart level for 15 minutes. In both the supine and SR positions, BP was measured four times using a non-invasive, standard inflatable cuff and a sphygmomanometer placed on the right arm. The last three were recorded and averaged to determine systolic BP (SBP), diastolic BP (DBP), and mean arterial pressure (MAP). A stethoscope was placed over the brachial artery pulse, below the bottom edge of the cuff (2 cm above the cubital fossa). Correct measurement of BP requires an appropriate sized cuff; each child was fitted with either a pediatric or adult cuff based on arm size.[26] MAP was calculated using the formula $MAP = 1/3 \cdot SBP + 2/3 \cdot DBP$.

Beat-by-beat RRI and BP

Following 15 minutes of supine rest, five minutes of beat-by-beat RRI and BP data were collected simultaneously. Beat-by-beat BP (SBP and DBP) was collected using photoplethysmography (Nexfin, BMEYE, Amsterdam, The Netherlands) from the middle finger of the left hand. Since BP taken at the finger slightly differs from that taken at the arm, the average manual BPs taken at the beginning of the beat-by-beat recording were used to adjust the beat-by-beat values collected simultaneously with photoplethysmography. Pulse pressure from the finger (PP_{Finger}) was calculated as the difference between SBP and DBP for a peripheral measure of PP. RRI was collected

using a standard single-lead electrocardiogram. Both BP and RRI were sampled at a rate of 1000 Hz, providing a basic resolution of 1 millisecond (ms). These beat-by-beat data were then used for BRS analysis. HR was calculated by dividing RRI (sec) into 60.

Carotid Sonography and Tonometry

Arterial images were obtained using high-resolution ultrasound sonography (Vivid q, GE Medical Systems, The Netherlands). Three 2-dimensional B-mode images were recorded over five cardiac cycles at a frame rate of 37 frames/sec using a 12MHz linear array transducer, for offline analysis. Two of the best quality images were used for determining arterial diameter (AD) measurements in systole and diastole. Three cardiac cycles were chosen for each of the two images. Carotid sonography was completed for the CCA 1-2cm proximal to the bifurcation, as well as the CS at the proximal portion of the ICA distal to the carotid bifurcation. The proper protocols and techniques for standardized carotid ultrasound were employed as recommended.[27, 28]

In addition, simultaneous applanation tonometry was used to non-invasively determine left CCA PP (PP_{CCA}) and CS PP (PP_{CS}). A hand-held tonometer (Millar Instruments, Houston Texas) was used to manually obtain pressure waveforms from the left CCA and CS. The transducer was calibrated with an external device utilizing a two-point calibration system. The pressure wave obtained is very similar to that recorded within the artery.[29, 30] Since the pressure required to applanate the artery and compress the overlying structures varies, the absolute values of systolic and diastolic pressure are not reliable, but the amplitude (PP) can be determined reliably.[20] Accuracy of the Millar devices at the sites of the carotid and radial arteries has been established.[29, 30] In order to obtain an acceptable waveform the criteria outlined by Chen et al., (1996)[30] were

followed so that the operator adjusted the hold-down pressure so that acceptable waveforms had a stable baseline, maximum amplitude, and a reasonable configuration. Tonometry was completed for both the CCA and the CS in both the supine and SR position. The same researcher completed all image and tonometric acquisition, as well as analysis. Sample ultrasound images and corresponding pressure waveforms for the CCA (Appendix A) and CS (Appendix B) are provided.

Data Analysis

Cardiovagal BRS

Beat-by-beat data were sampled at 1000 Hz using an online data analysis and acquisition system (Powerlab and Chart 7 PRO, ADInstruments). Data were saved for offline analysis and scanned to ensure that it was free from ectopic beats. Matlab (Mathworks, R2012b) was used to resample the data using the mean cardiac frequency to obtain an equal interval between samples. A low-pass Butterworth filter set to 0.95 Hz was used and the data was detrended. For transfer function analyses, Fast Fourier Transform (FFT) was used with the Welch method and Hanning window, with the window size set to one-fourth of the signal length with one-half overlap. LF area was set to 0.05-0.15 Hz. Mean transfer function gain was used to determine BRS for the LF region using a coherence ≥ 0.5.

Arterial Distensibility

Three maximum (systolic) and minimum (diastolic) diameters were measured for three cardiac cycles per image, using edge-detection software software (Artery Measurement System II, Image and Data Analysis) in a specified region of interest (ROI). The software identifies the artery wall within the ROI based on the contrast of brightness

and intensity between the wall and lumen. Arterial diameter was measured at the leading edge of adventitial-medial border of the near wall to the medial-adventitial border of the far wall. Diameters were measured at the level of the CCA and CS. The landmark for measurements at the CCA was 2cm proximal to the carotid bifurcation. The CS was landmarked at the proximal root of the ICA, just distal to the carotid bifurcation.

Arterial distensibility coefficient (DC) was calculated at the CCA (DC_{CCA}) and CS (DC_{CS}) using the standard equation:[31-33]

$$DC\ (mmHg^{-1} \times 10^{-3}) = (\Delta CSA) / (PP \cdot CSA_{min}) \qquad (1)$$

Cross-sectional area (CSA) was calculated as $CSA = \pi r^2$, where r = diameter/2 for AD. Distension (ΔCSA) was calculated as $\Delta CSA = CSA_{max} - CSA_{min}$. PP was taken as the pressure increase from diastole to systole ($PP = P_s - P_d$) averaged from 10-15 cardiac cycles using applanation tonometry for CCA (PP_{CCA}) and CS (PP_{CS}). Strain (%) was calculated as strain (%) = $\Delta CSA/CSA_{min}$ for both the CCA ($Strain_{CCA}$) and CS ($Strain_{CS}$).

Statistical Analysis

All statistical analyses were completed using SPSS software (IBM SPSS Statistics 20). Descriptive statistics of demographic, anthropometric, and cardiovascular variables are expressed as mean ± standard deviation (SD) for continuous variables and as frequencies for categorical variables. The level of significance was set to $p<0.05$ (two-tails). Chi-square was used to test the difference in proportion of males and females in the sample.

Paired t-tests were used to compare differences in arterial properties between the CCA and CS, and to test the effect of the posture stimulus on arterial properties, as well as BRS. Pearson's correlation was used to examine the relationship between strain, PP,

and DC measured at the CCA and CS for supine. Relative change variables were created for systolic and diastolic diameters, strain, PP, and DC, for both the CCA and CS, as well as for BRS. Differences in responses to the SR posture between the CCA and CS were determined using paired t-tests for systolic and diastolic diameters, strain, PP, and DC. Pearson's correlation was used to examine the relationship between relative change in strain, PP, and DC for the CCA and CS. Furthermore, regression analysis was used to examine the relationship between relative change in BRS (ΔBRS) and relative change in DC_{CCA} (ΔDC_{CCA}), $Strain_{CCA}$ ($\Delta Strain_{CCA}$), DC_{CS} (ΔDC_{CS}), and $Strain_{CS}$ ($\Delta Strain_{CS}$). The known effects of maturation and sex on BRS(Chirico)[18] were controlled for in regression analyses. Three models were created: Model 1 included ΔBRS as the dependent and one of the arterial properties for CCA and CS (ΔDC, ΔStrain, and ΔPP) as the independent variable. Model 2 controlled for sex, and Model 3 controlled for maturation.

All variables were normally distributed in the supine and SR positions. Change variables were used to test the assumption of normality for paired. Only relative change in $Strain_{CCA}$ was not normally distributed. Log transformation and nonparametric testing did not change the results for $Strain_{CCA}$.

Results

Twelve participants were included in the study (4 males and 8 females) ranging from 11.4-17.4 years of age. The distribution of males and females was not different (χ^2 =1.33, df=1, ns). Further demographic and anthropometric data are presented in Table 7-1. The cardiovascular responses to SR posture are presented in Table 7-2. Compared with supine, DBP ($p<0.001$), MAP ($p<0.001$) and HR ($p<0.01$) increased in the SR posture.

Table 7-1. Participant demographic and anthropometric information

N	12
Age (years)	14.4 ± 2.1
Sex (% male)	33%
Height (cm)	164.3 ± 13.1
Body Mass (kg)	54.5 ± 15.4
BMI (kg/m^2)	19.8 ± 4.0
Maturation	
Pre	2
Early	2
Peri	0
Late	6
Post	2

Mean ± SD. BMI=body mass index. Pre=pre-pubertal, Early=early-pubertal, Peri=peri-pubertal, Late=late pubertal, and Post=post-pubertal.

Table 7-2. Cardiovascular responses from supine to seated-recumbent

	Supine	SR
SBP (mmHg)	109 ± 8	109 ± 10
DBP (mmHg)	60 ± 6	71 ± 4$^{\tau}$
MAP	76 ± 5	84 ± 4$^{\tau}$
HR (beats/min)	68 ± 10	73 ± 11*

Mean ± SD. SR=seat-recumbent, SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; HR=heart rate. Note: Paired T-test used to compare supine versus seated-recumbent *$p \leq 0.01$, $^{\tau}p \leq 0.001$

CCA and CS arterial properties in supine and SR postures

Table 7-3 presents the means and SD for arterial properties at the CCA and CS in both the supine and SR position. There were no differences between systolic (-0.20 ± 0.76 mm, $t_{(11)}$=-0.91, p=0.384) and diastolic (-0.11 ± 0.63 mm, $t_{(11)}$=-0.58, p=0.576) diameters between the CCA and CS in supine. Furthermore, Strain (-2.9 ± 7.4 %, $t_{(11)}$=-1.37, p=0.197), PP (-4 ± 9.0 mmHg, $t_{(11)}$=-1.60, p=0.139), and DC (-0.04 ± 2.5 $mmHg^{-1}$ x 10^{-3}, $t_{(11)}$=-0.05, p=0.959) values also did not differ between the CCA and CS in the supine position. Pearson's correlation demonstrated a significant relationship between DC_{CCA} and DC_{CS}, but not for $Strain_{CCA}$ and $Strain_{CS}$ or PP_{CCA} and PP_{CS} (Table 7-4).

CCA systolic (0.36 ± 0.16 mm, $t_{(11)}$=7.91, p<0.001) and diastolic (0.37 ± 0.16 mm, $t_{(11)}$=7.80, p<0.001) diameters decreased in the SR position, while $Strain_{CCA}$ (-2.0 ± 4.4%, $t_{(11)}$=-1.6, p=0.138) and DC_{CCA} (0.8 ± 2.2 $mmHg^{-1}$ x 10^{-3}, $t_{(11)}$=1.23, p=0.244) remained unchanged (Table 7-3). CS systolic (0.50 ± 0.18 mm, $t_{(11)}$=9.48, p<0.001) and diastolic diameters (0.34 ± 0.20 mm, $t_{(11)}$=5.83, p<0.001), $Strain_{CS}$ (4.5 ± 5.0%, $t_{(11)}$=3.13 p=0.01), and DC_{CS} (2.3 ± 1.9 $mmHg^{-1}$ x 10^{-3}, $t_{(11)}$=4.21, p=0.001) decreased in the SR posture. PP_{CCA} increased (-5 ± 4 mmHg, $t_{(11)}$=-4.12, p=0.002), PP_{Finger} decreased (12 ± 7 mmHg, $t_{(11)}$=8.18, p<0.001), and PP_{CS} did not change (-2. ± 5 mmHg, $t_{(11)}$=-1.49, p=0.165) from supine to SR.

Table 7-3. Arterial responses from supine to seated-recumbent

	Supine	SR
sD_{CCA} (mm)	6.49 ± 0.53	6.13 ± 0.45$^{\tau}$
dD_{CCA} (mm)	5.72 ± 0.51	5.36 ± 0.45$^{\tau}$
$Strain_{CCA}$ (%)	29.1 ± 8.7	31.1 ± 6.9
PP_{CCA} (mmHg)	29 ± 6	33 ± 5*
DC_{CCA} ($mmHg^{-1}$ x 10^{-3})	10.3 ± 2.7	9.5 ± 2.1
sD_{CS} (mm)	6.69 ± 1.02	6.20 ± 0.93$^{\tau}$
dD_{CS} (mm)	5.83 ± 0.90	5.49 ± 0.84$^{\tau}$
$Strain_{CS}$ (%)	32.0 ± 6.0	27.5 ± 5.0*
PP_{CS} (mmHg)	33 ± 9	35 ± 7
DC_{CS} ($mmHg^{-1}$ x 10^{-3})	10.3 ± 2.9	8.1 ± 1.7$^{\tau}$
PP_{Finger} (mmHg)	51 ± 11	39 ± 11$^{\tau}$
BRS (ms/mmHg)	18.5 ± 9.2	17.1 ± 6.9

Mean ± SD. sD_{CCA}=common carotid systolic diameter; dD_{CCA}=common carotid diastolic diameter; $Strain_{CCA}$=common carotid strain; PP_{CCA}=common carotid pulse pressure; DC_{CCA}=common carotid distensibility coefficient; sD_{CS}=sinus systolic diameter; dD_{CS}=sinus diastolic diameter; $Strain_{CS}$=sinus strain; PP_{CS}=sinus pulse pressure; DC_{CS}=sinus distensibility coefficient; PP_{Finger}=finger pulse pressure. Note: Paired t-test used to compare supine versus seated-recumbent *$p \leq 0.01$, $^{\tau}p \leq 0.001$

Table 7-4. Pearson's correlations of arterial properties of the common carotid artery compared to the carotid sinus

Supine	r	p-value
Strain (%)	0.547	0.066
PP (mmHg)	0.337	0.284
DC ($mmHg^{-1}$ x 10^{-3})	0.618	**0.032**
SR		
ΔStrain (%)	0.035	0.914
ΔPP (%)	0.163	0.613
ΔDC (%)	0.353	0.260

PP=pulse pressure; DC=distensibility coefficient; SR=seated-recumbent; ΔStrain (%)=relative change in strain; ΔPP (%)=relative change in pressure; ΔDC (%)=relative change in distensibility coefficient

Figure 7-1 illustrates the differences in responses between the CCA and CS to SR posture. There was no significant difference in response between the CCA and CS for systolic (2 ± 3%, $t_{(11)}$=2.10, p=0.06) and diastolic (-1 ± 5%, $t_{(11)}$=-0.50, p=0.683) diameters, or PP (8 ± 22, $t_{(11)}$=1.27, p=0.231;Figure 7-1A-C). There was a significant difference in response for Strain (23 ± 24%, $t_{(11)}$=3.34, p=0.007) and DC (14 ± 20, $t_{(11)}$=2.46, p=0.032; Figure 7-1D and E). Furthermore, Pearson's correlation of the relative change in DC, Strain, and PP between the CCA and CS are presented in Table 7-4. There were no significant correlations between the CCA and CS for any of these variables.

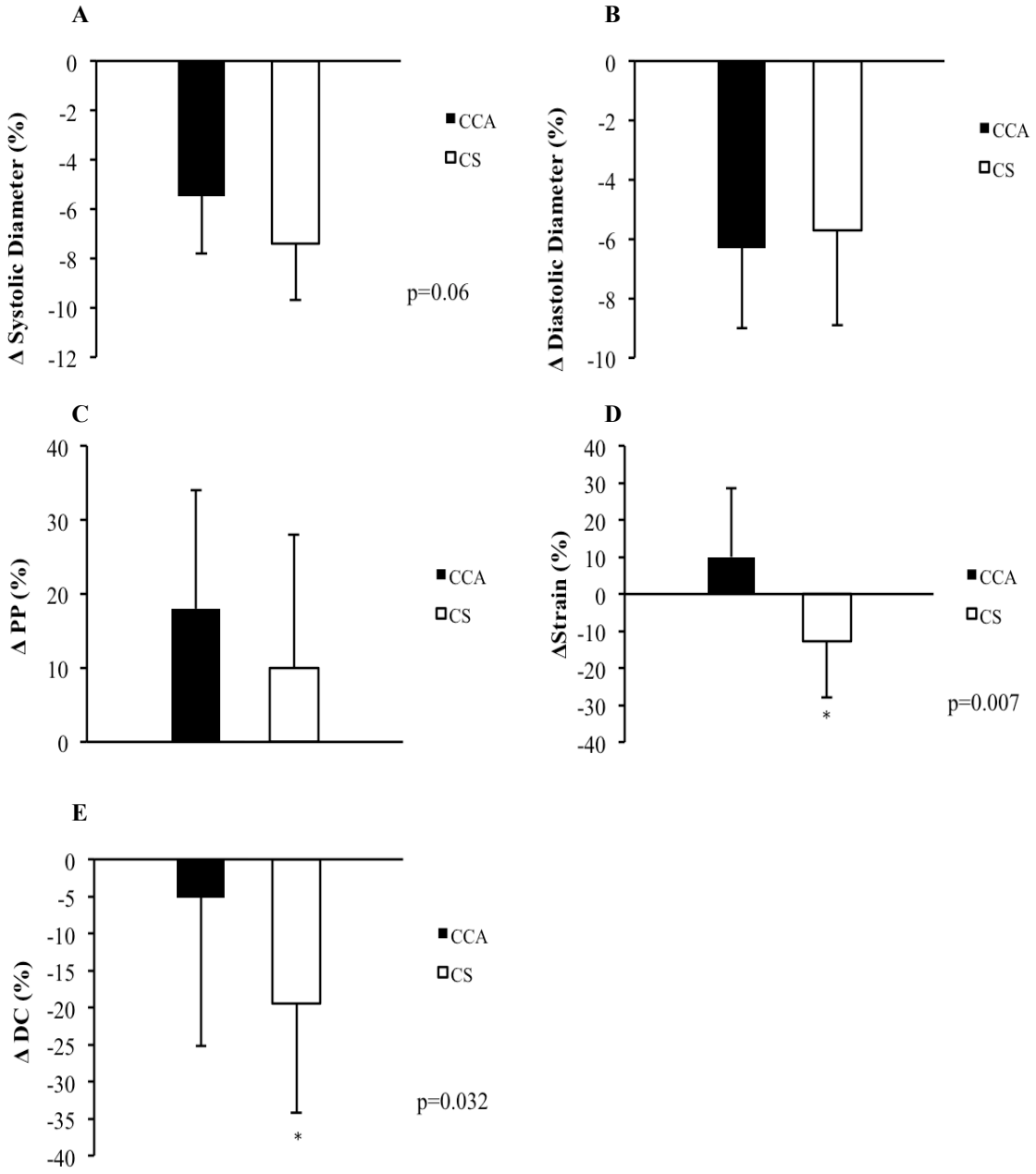

Figure 7-1
Comparison between the common carotid artery (CCA, black bars) and carotid sinus (CS, white bars) of the relative change in response to seated-recumbent posture for A) systolic diameter; B) diastolic diameter; C) Pulse pressure (PP); D) Strain; and E) distensibility (DC). Values are present as mean ± SD. Note: *significantly different from CCA determined by paired t-test.

BRS response to SR postural stimulus

There was no change in BRS (1.5 ± 4.7 ms/mmHg, $t_{(11)}$=1.07, p=0.306) in response to the SR posture (Table 7-3). Upon closer examination it was apparent that children and adolescents varied in response to SR. In 42% (5/12) BRS increased (Positive Responders, [PR]) in response to the SR posture, while 58% (7/12) decreased (Negative Responders, [NR]). The results are illustrated in Figure 7-2. In the NR group BRS decreased (4.3 ± 4.1ms/mmHg, $t_{(6)}$=2.78, p=0.032), while in the PR group BRS increased in the SR posture (-2.5 ± 1.5 ms/mmHg, $t_{(4)}$=-3.81 p=0.019). Supine BRS was not significantly different between the NR and PR groups (21.3 ± 8.3 vs. 14.6 ± 9.9 ms/mmHg, $t_{(10)}$=1.26, p=0.235). A low power (β=0.21) to detect a difference may account for this, as the effect size was moderate (0.73). Therefore, due to the large differences in BRS values and varying responses between individuals a relative change variable was used to examine the relationship between arterial distensibility, strain, PP, and BRS in response to SR.

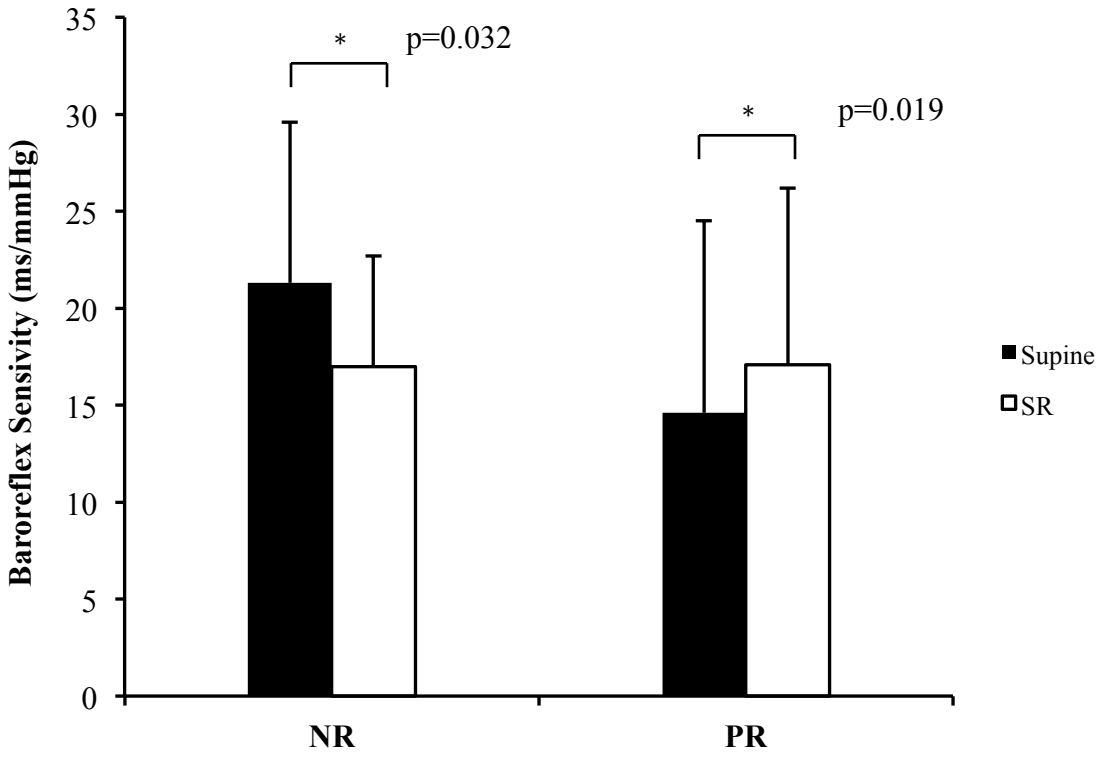

Figure 7-2
Baroreflex sensitivity responses to seated-recumbent (SR) posture in positive responders (PR) and negative responders (NR). Values presented as mean ± SD. Note: *Paired t-test SR significantly different from supine.

CCA Distensibility, Strain, PP and BRS

Regression analysis demonstrated that relative change in DC_{CCA} (Figure 7-3A) was not associated with relative change in BRS (B=-0.077, R^2=0.002, p=0.892). Moreover, relative $Strain_{CCA}$ change (B=-0.194, R^2= 0.01, p=0.752) was not related to relative change in BRS (Figure 7-3B). Additionally, relative change in PP_{CCA} (B=-0.142, R^2=0.004, p=0.839) was not a significant predictor of relative change in BRS. Results remained the same after controlling for sex and maturation.

DC_{CCA} was also examined by replacing PP_{CCA} with PP_{Finger} to compare to previous studies. The relationship between relative change in BRS and relative change in DC_{CCA} remained unrelated (B=-0.453, R^2=0.033, p=0.573).

CS Distensibility, Strain, PP and BRS

Relative change in DC_{CS} (Figure 7-4A) correlated significantly with relative change in BRS (B=1.807, R^2=0.573, p=0.004). Furthermore, relative change in $Strain_{CS}$ (Figure 7-4B) was correlated with relative change in BRS (B=1.597, R^2=0.483, p=0.012). Relative change in PP_{CS} (B=-0.077, R^2=0.002, p=0.892) was not correlated with relative change in BRS. Results remained the same after controlling for sex and maturation.

When PP_{Finger} was used to replace PP_{CS} for distensibility calculation, relative change in DC_{CS} was no longer related to relative change in BRS (B=0.586, R^2= 0.192, p=0.154).

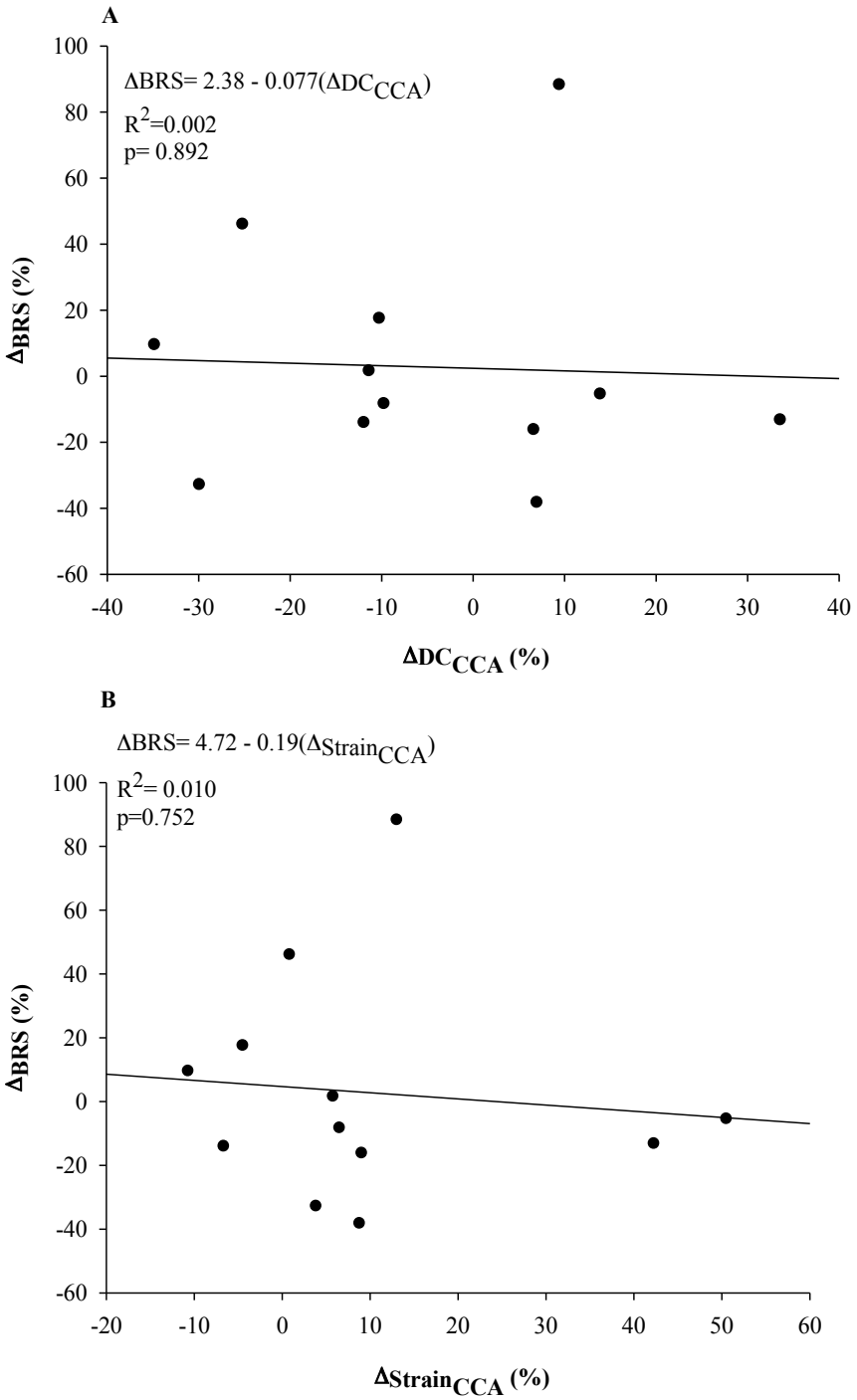

Figure 7-3
A) Regression analysis of the relative change in baroreflex sensitivity (BRS) on the relative change in common carotid distensibility (DC_{CCA}); B) Regression analysis of the relative change in BRS on the relative change in common carotid Strain ($Strain_{CCA}$).

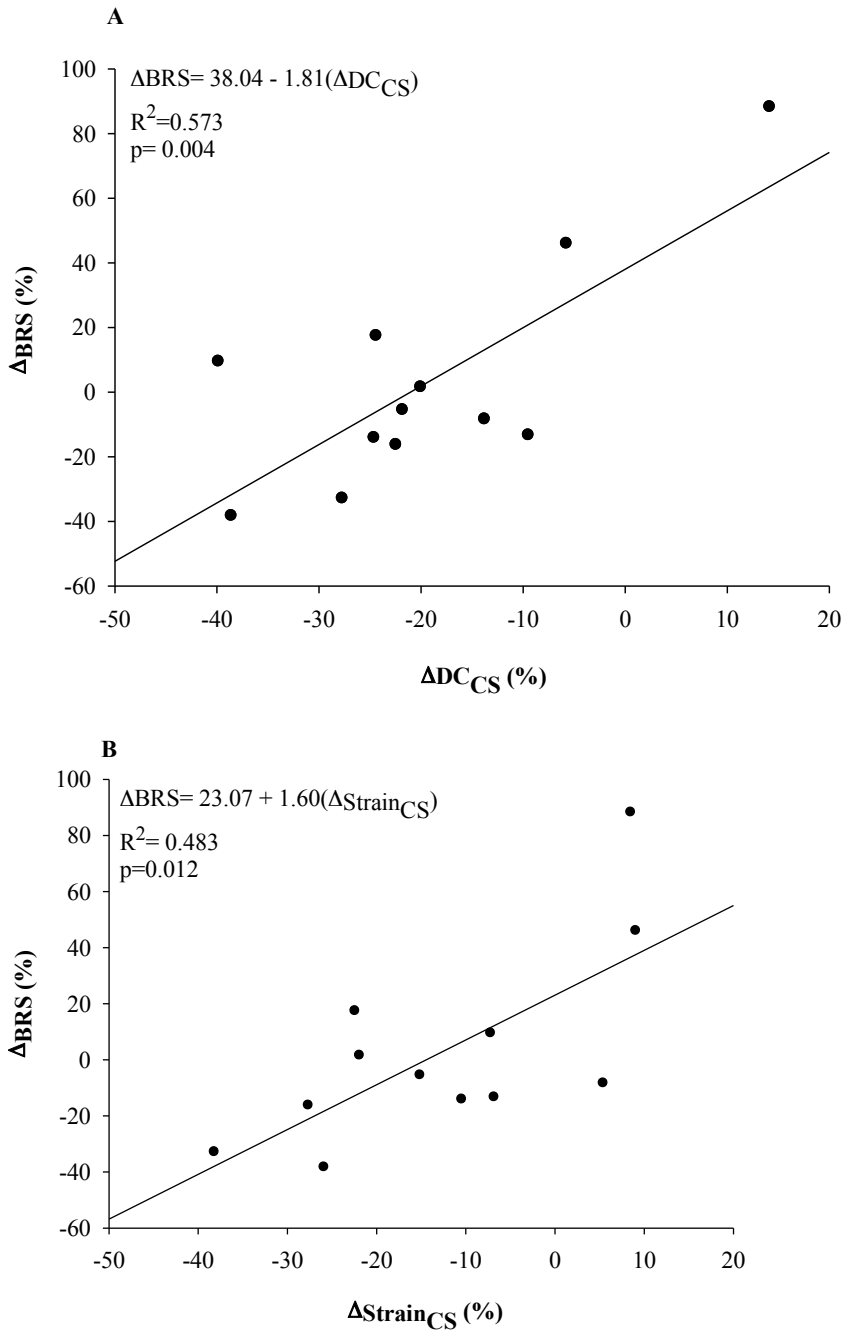

Figure 7-4
A) Regression analysis of relative change in baroreflex sensitivity (BRS) on relative change in carotid sinus distensibility (DC_{CS}); B) Regression analysis of relative change in BRS on relative change in carotid sinus strain $Strain_{CS}$.

Discussion

This was the first study to examine the relationship between arterial properties and BRS in response to posture in children and adolescents. This was also the first study to compare arterial properties between the CCA and CS and their relationship to BRS. The novel finding of the present study was that the relative change in BRS in response to a moderate postural stimulus was correlated with the relative change in DC_{CS} (r=0.757) and $Strain_{CS}$ (r=0.695). Furthermore, DC and Strain responded differently in response to the postural stimulus in the CS compared to the CCA. Overall, the present data indicate that DC_{CS} and $Strain_{CS}$ play an important role in baroreflex regulation in children and adolescents. These findings support the hypothesis of the importance of arterial properties on BRS in response to a postural stimulus and highlight the value of assessing arterial properties of the CS over the CCA.

Few studies have examined the effect of orthostatic stress on BRS in children and adolescents. Dietrich et al., (2006)[18] found that active standing decreased BRS significantly in boys and girls (age range 10-13 years). In comparing our BRS values with theirs in the supine position, ours are slightly higher which may be expected given the difference in age range. Our BRS values in the supine position are in line with those of a similar age range from Lenard et al., (2004)[16]. Discrepancies may be attributed to differences in maturation for children and adolescents of the same age range, as well as slight differences in methodologies in determining BRS. Dietrich found a more pronounced decrease in BRS in response to active standing. The reason for this may be the strength of the stimulus, as active standing provides a considerably greater stimulus than a 50° SR posture. However, the HR and BP responses to SR in the present study are

in line with studies in adults demonstrating an increase in HR and DBP, and no change in SBP.[15, 34]

The response to SR varied among participants with some demonstrating a decrease in BRS, while others demonstrated an increase. This observation is consistent with previous studies that have found BRS to decrease,[10, 11, 15, 17, 35] remain unchanged, [34] or increase[36] in response to orthostatic stress. When the data were split by NR and PR both groups demonstrated strong positive correlations between relative change in BRS and relative change in DC_{CS} (data not shown). Elucidating a mechanism to the heterogeneity in BRS responses is beyond the scope of this study. Previous reports suggest that an interaction between the cardiopulmonary and arterial baroreceptors may be responsible for augmented cardiovagal BRS.[36-40] It is possible that the PR had selective unloading of the cardiopulmonary baroreflex, which resulted in augmented vagal functioning in response to the postural stimulus as a result of eliminating an inhibitory effect of the cardiopulmonary baroreflex on the arterial baroreflex.[39] Additionally, unloading cardiopulmonary baroreceptors increases SNS activity,[37] which may enhance baroreceptor sensitivity. A study by Landgren (1952)[4] found that increased sympathetic stimulation to the carotid sinus in experimental preparations enhanced baroreceptor sensitivity.

The importance of arterial mechanics on cardiovagal BRS has been demonstrated in adults.[13, 14] O'Leary et al., (2005)[14] showed that increased distension of the CCA in response to phenylephrine was associated with increased cardiovagal BRS. Additionally, Kornet et al., (2002)[13] demonstrated that relative distension of the CCA was a better predictor of RRI variability than peripheral PP. This concept also applies to findings of

diminished BRS in response to orthostatic stress in adults, which has been attributed to reductions in the arterial mechanical component.[11, 15] In contrast, a recent study by Taylor et al., (2013)[10] found that a decrease in BRS in response to orthostatic stress was a result of reductions in the neural component. Disparities in findings have been linked to the methodological differences used to determine BRS and its components.[10] An additional limitation is the method in which the arterial mechanical component is quantified. Previous studies have evaluated the arterial component by examining the CCA and using peripheral BP.[6, 7, 10, 11] Carotid baroreceptors have been localized to the medial portion of the CS, distal to the carotid bifurcation.[19] These receptors are innervated by the carotid sinus nerve, which has been shown to insert loosely onto the ICA, ending at the level of the carotid bifurcation.[41] Moreover, it has been shown that peripheral PP does not reflect central PP responses to HUT;[20] a finding we have extended to children and adolescents in the SR position (Chapter 6). In addition, we found PP_{CS} to be greater than PP_{CCA}. Therefore, examining the CS directly may provide a better representation of the importance of arterial mechanics on BRS. One study demonstrated that alterations in CS distensibility occur prior to that of the CCA with aging and borderline hypertension,[21] and another study found a positive correlation between CS distensibility and BRS at rest ($r=0.7$, $p<0.01$) and in response to phenylephrine ($r=0.72$, $p<0.01$), in adults.[42] This is particularly important in children and adolescents as the CS demonstrates growth during adolescence, whereas the CCA does not.[22] In the present study CCA distensibility did not differ from CS distensibility in supine, but their responses to the SR posture were different. Furthermore, the relative change in DC_{CS} was a significant predictor of the relative change in BRS. Hence, examining the CCA alone would have provided

erroneous conclusions to the relationship between arterial mechanics and BRS in response to a postural stimulus. To further emphasize the importance of measurement site, distensibility measures were also determined using PP_{Finger} in lieu of PP_{CS} and PP_{CCA}, as used in previous studies. Although results did not change with respect to DC_{CCA}, the relative change in DC_{CS} in response to SR posture was no longer correlated with the relative change in BRS.

In the present study relative change in $Strain_{CS}$ and DC_{CS} explained 48% and 57% of the relative change in BRS, respectively. However, the role of aortic baroreceptors was not tested and its importance cannot be ignored. Previous studies have demonstrated that increased aortic stiffness, assessed by pulse wave velocity, is associated with decreased BRS.[43, 44] Furthermore, one study examined the relationship between CS distensibility and aortic arch distensibility to BRS and determined that aortic distensibility was a more important predictor of BRS.[45] Moreover, the present study did not quantify the effect of the neural component on BRS and its impact cannot be discounted.[10]

Limitations: First, the use of a moderate postural stimulus in the present study may not have provided as strong of a stimulus in comparison to other studies.[10, 15, 18] However, it is believed that the stimulus was sufficient in children and adolescents as indicated by the significant changes in BRS and arterial mechanics associated with the stimulus. A seated posture has been used previously in adult studies.[11] Secondly, this study assessed CS diameters and PP in order to determine distensibility. There is considerable technical skill required to image the CS due to anatomical variations of the carotid bifurcation. Also, the proximity of the CS to the jawline in young children and adolescents can interfere with the acquisition of images at this location. These anatomical

constraints explain the small sample size. Thirdly, caution must be taken when determining diameters of the CS due to its ellipsoid shape. Arterial diameters and distensibility have been shown to differ at various segments of the CS.[21, 46] With this in mind, the authors used stringent criteria to consistently measure arterial diameters at the proximal portion of the ICA, just distal to the bifurcation. This was chosen based on the fact that baroreceptors are localized to this portion of the sinus.[19, 41]

In conclusion, the findings of the present study highlight the importance of measuring CS distensibility when evaluating the relationship between arterial distensibility and BRS in response to a postural stimulus. Although measures of arterial properties between the CCA and CS were significantly correlated in the supine position, they varied significantly in their response to the SR posture. The present study demonstrated that the relative change in $Strain_{CS}$ and DC_{CS} were significantly correlated to the relative change in BRS in response to a postural stimulus, whereas $Strain_{CCA}$ and DC_{CCA} were not. These findings have important methodological implications in the assessment of the arterial mechanical component of the baroreflex.

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Chapter 8: General Discussion

8.1 Summary of Major Findings

An overview of major findings is depicted in Figure 8-1. Results from Chapter 4 (study 1) provided important new information regarding the effect of sex and maturation on BRS. The major finding was a sex-by-maturation interaction whereby males demonstrated a decrease in BRS from early- to post-puberty, while BRS for females remained unchanged. This was associated with a sex difference post-puberty. These findings were in contrast to our hypothesis that BRS would improve with maturation, but supports the hypothesis that maturation is important to consider when examining BRS in children and adolescents. Age, height, body mass, BMI, SBP, and MAP were significant correlates of BRS but they did not explain the sex-by-maturation interaction when included in the ANCOVA analyses. Age was only weakly correlated with BRS in males. Importantly, anthropometric and cardiovascular changes with puberty are supported by our results. We found height, body mass, BMI, FFM, and FM to increase with maturation.

In order to elucidate an explanation for the sex-dependent maturation effect on BRS, Chapter 5 (study 2) examined the relationship between maturation and CCA distensibility, as well as the relationship between CCA distensibility and BRS. To our knowledge there was no study assessing maturation and CCA distensibility across all stages of pubertal maturation at the onset of this PhD project (2010). Given that arterial distensibility is an important component of BRS, it was hypothesized that the sex-by-maturation interaction would be explained by a similar pattern in CCA distensibility. This was further supported by previous findings by Ahimastos et al., (2003)[1] who compared central PWV in pre- and post- pubertal boys and girls. Their results demonstrated a sex-

by-maturation interaction in which girls demonstrated a significant decrease in central arterial stiffness from pre-to-post-puberty, while males demonstrated an increase. Based on the findings from Chapter 4, and those reported by Ahimastos, we hypothesized that CCA distensibility would change with maturation in a similar pattern as BRS. Specifically, it was hypothesized that males would demonstrate a decrease in CCA distensibility, whereas females would demonstrate unchanged or increased distensibility. In this study we found that CCA distensibility did not change with maturation in males or females; it remained stable throughout. Furthermore, CCA diameters and IMT also remained stable throughout maturation. There were sex differences evident in CCA maximum and minimum diameters, as well as IMT. There was no interaction between sex and maturation. Additionally, there was no association between CCA distensibility and BRS. These findings suggest that the observations in Chapter 4 may be explained by neural alterations of the baroreflex, as there was no relationship between CCA distensibility and maturation or BRS in Chapter 5. The disparities between the findings of Ahimastos et al., (2003)[1] and ours may be the result of different measurement techniques encompassing different segments of the arterial tree. CCA distensibility is a measure of local arterial stiffness, while PWV is a measure of regional stiffness.[2] Regional measures of stiffness incorporate multiple arterial segments containing varying degrees of stiffness; therefore, relating regional and local measurements of stiffness may not be accurate.[2, 3] Studies in adults have shown the aorta stiffens to a greater degree than the CCA with age and in the presence of cardiovascular risk factors.[4-6] Hence, the aorta may be more susceptible to maturation or age-related changes than the carotid artery. There is also evidence demonstrating that increased stiffness can be detected in the CS prior to the

CCA with respect to aging and cardiovascular disease risk factors in adults.[7] This provides support for assessing the CS over the CCA when examining the effect of arterial distensibility on BRS.

To further examine the importance of the arterial component on BRS, Chapters 6 (study 3) and 7 (study 4) aimed to determine whether assessing the CCA was suitable to examine the relationship between arterial distensibility and BRS in response to orthostatic stress. In Chapter 6 we tested the hypothesis that central arteries (CCA and CS) respond differently than peripheral arteries (Finger), with respect to PP, to orthostatic stress. Secondly, we hypothesized that PP in the CS would differ from that of the CCA. Our findings demonstrated a significant decrease in PP at the periphery site (Finger), but not centrally (CCA, CS), in response to SR. The PP at the CS was also significantly greater than the CCA. These findings suggest that assessing arterial properties at the CS might prove to be useful when assessing the relationship between arterial properties and BRS. Chapter 7 aimed to address the question of whether CS distensibility is a better predictor of BRS than the CCA in response to orthostatic stress. In this study we used ultrasound imaging of the CCA and CS, as well as tonometry at each location, to determine distensibility in the supine and SR positions. We found that the relative change in distensibility at the CS was significantly and positively correlated to the relative change in BRS in response to SR. This was not the case for the CCA. Furthermore, relative $Strain_{CS}$ change was also significantly correlated with relative change in BRS. These findings support the hypothesis that examining the CS is more suitable to assess the relationship between arterial mechanical properties and BRS.

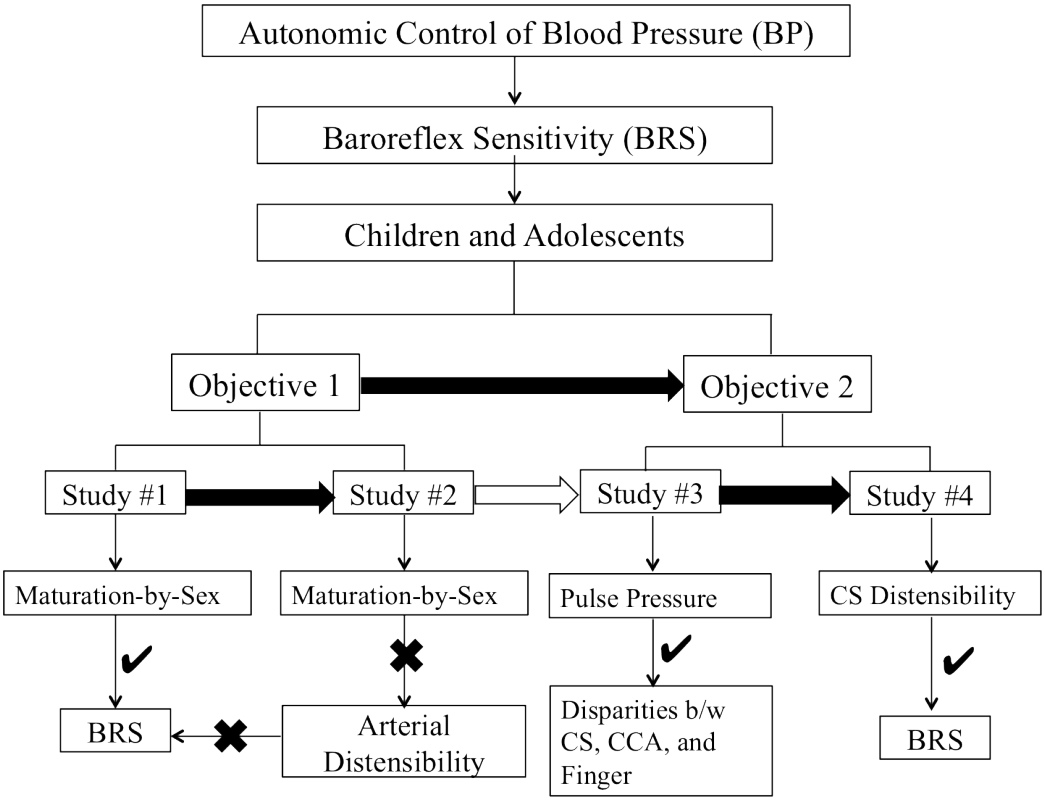

Figure 8-1
Overview of major novel findings

8.2 Maturation and Sex

8.2.1 Baroreflex Sensitivity

As indicated previously, the findings from study 1 demonstrated a significant sex-by-maturation interaction whereby BRS decreased in males from early- to post-puberty, but remained unchanged in females. This resulted in a sex difference in the Post group with females having greater BRS than males. Baroreflex function is reduced in children and adolescents with cardiovascular risk factors such as obesity,[8-11] and high BP.[12-14] The significance of this diminished BRS can only be understood in comparison to normal healthy development. Furthermore, it is not known how diminished BRS at a young age can impact BRS and cardiovascular risk, in adulthood. The findings of the present study contribute to our understanding of the importance of maturation on autonomic function, as well as the importance of considering pubertal maturation when evaluating BRS in children and adolescents. There is only one study that we are aware of examining pubertal maturation on BRS, [15] while others have used age to assess the development of BRS.[16, 17]

Our findings are in contrast with previously reported studies in children and adolescents. In an attempt to assess the maturation of cardiovagal BRS Lenard et al., (2004)[17] examined children, adolescents, and young adults from 7-22 years old and categorized them into age groups. BRS increased with increasing age group from 7-18 years old with no further change in the 18-22 year olds. This study is limited by the use of age, not puberty, to assess maturation of BRS. In contrast, Zavodna et al., (2008)[16] found that BRS was not related to age in children and adolescents 11-22 years old. Only one study by Dietrich et al., (2006)[15] has examined the effect of puberty on BRS, in a large sample of 10-13 year olds, by categorizing individuals into pubertal groupings using

the criteria of Tanner. Although no maturation effect on BRS was found in the supine position, this study failed to include a full scope of pubertal maturation as it clustered a number of pubertal stages into a single grouping. Therefore, our findings are the first to demonstrate the importance of maturation and sex on BRS in children and adolescents.

Elucidating a mechanism to the sex-by-maturation interaction effect is beyond the scope of this particular study. While a majority of studies examining sex differences in BRS in adults have found males to have significantly greater BRS than females,[18-22] this finding is not universal.[23] Reports of sex differences in children and adolescents are equivocal.[15-17] Our findings demonstrated that a sex difference emerges in the Post group and highlights the importance of maturation status in determining sex differences in children and adolescents. Furthermore, our findings suggest a possible sex hormone influence on BRS development. The beneficial effect of testosterone and estrogen on BRS has been demonstrated in both animals and adults.[24-27] The present study did not measure sex hormones and cannot draw conclusions on their possible impact on the present findings. Future work in children and adolescents regarding the impact of sex hormones on BRS development is required.

An important component of baroreflex function is the arterial mechanical component. Several studies have demonstrated the important relationship between arterial distensibility and BRS in adults.[28-33] The sex-by-maturation effect found in Chapter 4 may be a reflection of differential effects of maturation on arterial distensibility in males and females. Chapter 5 (study 2) aimed to examine the effect of maturation on CCA distensibility, and the relationship between CCA distensibility and BRS.

8.2.2 Common Carotid Distensibility

In the present study there was no evidence of a maturation or sex effect on CCA distensibility. Similarly, there were no changes in CCA maximum or minimum diameters throughout maturation. The findings of the present study indicate that the sex-by-maturation interaction found in Chapter 4 cannot be explained by changes in CCA distensibility. Our findings are consistent with two recent reports in the literature with regards to maturation, sex, and CCA distensibility. Marlatt et al., (2013)[34] evaluated CCA distensibility in a large cohort of children and adolescents and grouped them into pubertal stages based on the criteria of Tanner. It was found that CCA distensibility remained stable throughout maturation. The authors concluded that controlling for pubertal maturation when evaluating CCA distensibility in children and adolescents is not necessary. In another study by Marlatt et al., (2013),[35] sex differences were not evident in CCA distensibility in children and adolescents.

In the present study we found that CCA distensibility was not related to BRS. Limited studies have examined the effect of arterial distensibility on BRS in children and adolescents. Lenard et al., (2004)[17] examined CCA distensibility and BRS in children, adolescents, and young adults aged 7-22 years old. They found that CCA distensibility decreased with age while BRS increased. Furthermore, there was no sex difference in CCA distensibility. A study by Pinter et al., (2007)[36] found that children and adolescents with surgically repaired transposition of the great vessels had decreased CCA distensibility compared to controls, but no difference in BRS. Furthermore, CCA distensibility did not correlate with BRS in either group. Both studies suggest neural adaptations maintain BRS in the presence of decreased CCA distensibility. The results of

our investigation also highlight the importance of neural alterations.

Our findings, as well as those by Marlatt et al., (2013a, 2013b),[34, 35] are in contrast to the findings of Ahimastos et al., (2003)[1] in which there was a sex-by-puberty interaction in aortic PWV. They found that PWV was greater in pre-pubertal girls compared to boys, but PWV increased in boys post-puberty (increased aortic stiffness) and decreased in girls, eliminating sex differences. The present study did not examine PWV and cannot draw conclusions based on its impact on BRS with respect to maturation. However, it has been shown by Lenard et al., (2001)[37] that aortic arch distensibility is a stronger predictor of BRS than CS distensibility in aging adults. Taken together, these studies suggest the possibility of aortic stiffness explaining the sex-by-maturation effect in Chapter 4. The importance of PWV on BRS has been demonstrated in adults.[28, 38] Future directions in children and adolescents should examine the role of aortic stiffness and maturation, and its effect on BRS. This may provide further insight into the maturation effect of BRS found in Chapter 4.

8.3 Arterial Stiffness and BRS

Several investigations have examined the importance of arterial properties on BRS.[28, 32, 39] Bonyhay et al., (1996)[33] examined the relationship between CCA distensibility and BRS in young healthy adults with a mean age of 22 years. They found a significant correlation between CCA distensibility and BRS (r=0.78). It was the sole predictor of BRS using forward stepwise regression and explained 61% of the variability in BRS. Monahan et al., (2001)[30] further highlighted the importance of arterial elasticity on BRS when examining the relationship between age, BRS, and CCA compliance in 47 healthy adults aged 19-76 years. There was a negative correlation between BRS and age

(r=-0.69), and a positive correlation between CCA compliance and BRS (r=0.71). Common carotid compliance was the strongest independent predictor of BRS and explained 51% of the variability. When partial correlation analysis was completed between age and BRS, controlling for CCA compliance, the relationship between age and BRS was weaker. An additional component to this study was a 3-month aerobic training program in 13 adults (>50 years of age). Cardiovagal BRS and CCA compliance improved over the exercise intervention by 27% and 29%, respectively. There was a strong positive relationship between the change in CCA compliance and cardiovagal BRS (r=0.72). The increase in CCA compliance explained 50% of the variability that was associated with the cardiovagal BRS improvement.[30] Together, the studies of Monahan and Bonyhay demonstrate the importance of CCA distensibility on BRS in healthy adults.

The interest in determining the importance of the arterial mechanical component on BRS versus the neural component led to the advanced methodology created by Hunt et al., (2001)[40] to quantitatively delineate each component. This technique utilizes drug-induced increases and decreases in BP while simultaneously recording CCA diameters, RRI, and SBP. This allows for the quantification of integrated BRS, as well as the individual mechanical and neural components. This method has been applied in various experimental conditions in order to elucidate the mechanism of reduced BRS that occurs with aging,[29, 41] hypertension,[42] and orthostatic stress.[31, 43] Various studies, using a variety of techniques, remain equivocal on the importance of one component over the other.[29-32, 39, 41, 43]

The importance of measurement site location was highlighted by Kornet et al., (2002)[44]. They illustrated that central measures of CCA distension, distension rate, and

relative distension explain the variability in RRI to a greater extent than peripheral measures of pressure. They suggested that in order to eliminate the bias that may arise from peripheral measurements, only the relationship between changes in CCA diameter to changes in RRI should be considered. The objective for Chapters 6 and 7 was to examine the relationship between arterial properties and BRS in response to orthostatic stress by comparing the CCA and CS. In Chapter 6 we examined the differences between PP_{CS}, PP_{CCA}, and PP_{Finger} to determine differences between sites in supine, and in response to postural change. We found that PP_{CS} was greater than PP_{CCA}, and that PP_{Finger} was greater than both PP_{CS} and PP_{CCA} in the supine position. These findings are important as they provide evidence that the use of PP_{Finger}, and even PP_{CCA}, may not accurately reflect local pressure in the CS. It was also found that PP_{Finger} decreased in response to posture change, while PP_{CS} and PP_{CCA} remained unchanged. This is also an important finding to consider when examining the relationship between arterial properties and BRS in response to orthostatic stress. Our findings are consistent with Steinback et al., (2004)[45] and extend their findings to the CS, as well as children and adolescents. With our previous findings in consideration, Chapter 7 aimed to determine the importance of CS distensibility on BRS in response to posture change. In this study, 12 children and adolescents (4 males and 8 females) were included. It was found that the relative change in CS distensibility was correlated (r=0.757) with the relative change in BRS from supine to SR posture; however, relative change in CCA distensibility was not. Furthermore, the relative change in $Strain_{CS}$ was correlated (r=0.669) with relative change in BRS, but $Strain_{CCA}$ was not.

A series of recent studies have provided support for the investigation of the CS

over the CCA. Two studies conducted by Toorop and colleagues[46, 47] have made contributions to the understanding of baroreceptor location and CS nerve innervation. They aimed to determine CS nerve anatomy using twelve human cadavers. It was determined that the CS nerve ran parallel to the ICA and always ended at the level of the bifurcation in a position of anteromedial (n=6/12), anterolateral (n=5/12) or anteriorly (n=1/12) in relation to the ICA; demonstrating a consistent innervation in the anterior portion. Distal portions of the CS nerve had numerous connections to the CS wall and/or carotid bifurcation. Their more recent study aimed to localize baroreceptor distribution in the CS.[47] Using a staining technique and light microscopy they localized staining to the adventitia, including the adventitia-media border. Positive staining was abundant in the medial portion of the CS with minimal or absent staining in the anterior and posterior portion. This was localized 1cm distal to the bifurcation with no staining evident in the CCA, external carotid artery, or 2 cm distal to the bifurcation. Together, these findings demonstrate the importance of examining the CS when determining the effect of arterial distensibility on BRS.

It is assumed that CCA distensibility represents CS distensibility; however, this may not be a correct assumption in aging, CVD risk factors, or even the maturing adolescent. With respect to aging and CVD risk factors, Van Merode et al., (1993)[7] examined strain and distensibility in the CS at its proximal, max (central), and distal locations versus the CCA in borderline hypertensive older adults (mean age 38 years) compared to age-matched normotensive controls, and normotensive young adults (mean age 24 years). Strain did not differ at any site in the young normotensives, but was significantly lower at the distal and proximal CS compared to the CCA in the

normotensive older group. Strain was lower at all parts compared to CCA in the borderline hypertensives. The proximal portion was also lower than the distal and max CS locations in this group. This is an important finding as baroreceptors are localized in the proximal portion of the CS.[47] Our findings of decreased $Strain_{CS}$ in the proximal ICA, and not $Strain_{CCA}$, in response to posture change are similar to the findings of Van Merode et al., (1993).[7] Furthermore, distensibility was decreased in the borderline hypertensives compared to age-matched controls at all locations except for the distal CS. In addition, an age-related change in distensibility between young and older adults was only seen at the sites of the CS. These findings suggest that alterations in CS distensibility occur prior to changes in the CCA, which supports our findings of the importance of examining the CS when assessing the relationship between arterial distensibility and BRS. Examining the CS is also of clinical relevance as carotid stenosis typically occurs in the body of the CS.[48, 49]

The importance of assessing the CS over the CCA when examining the effect of arterial distensibility on BRS is also applicable to children and adolescents. An interesting study conducted by Seong and colleagues [50] examined morphological characteristics of the carotid bifurcation from infancy to young adulthood (0-36 years). They found that the ICA was similar in size to the external carotid artery up to 9 years of age; however, between the ages of 10-19 there was significant growth at the root of ICA. Therefore, significant growth at the proximal root of the ICA occurs during adolescence. This further supports that importance of examining the relationship between CS distensibility and BRS in children and adolescents. This may potentially help explain the maturation related changes that occur with BRS.

8.4 Future Directions

The findings of the present thesis demonstrate the importance and necessity of examining autonomic cardiovascular control in children and adolescents. Based on the findings in Chapter 4 several future directions are necessary. We have demonstrated the importance of sex and pubertal maturation on BRS. To gain a better understanding of this relationship, a longitudinal assessment would be beneficial to follow children as they progress through puberty. This will allow for a more accurate representation of pubertal development. Examining sex hormone concentrations is also an important future step to understand the relationship between pubertal maturation and BRS. This will help to elucidate a mechanism for the relationship between maturation and BRS.

In order to determine the significance of early exposure to cardiovascular risk factors in children and adolescents and their impact on future autonomic development, tracking cardiovascular risk factors during this time period, and their impact on BRS, is necessary. This will allow for the identification of critical time periods in which exposure to risk factors have the greatest impact on autonomic development in children and adolescents. Furthermore, a better understanding of factors that may facilitate autonomic development is necessary. Currently, limited research exists examining the impact of physical activity on BRS in children and adolescents. A better understanding of the relationship between physical activity, pubertal maturation, and BRS in children and adolescents will provide a gateway to understanding critical time periods in which BRS can be improved in children and adolescents.

It is also necessary to further evaluate the impact of the arterial mechanical and neural components on BRS development in children and adolescents. The findings from

Chapter 5 implicate involvement of the neural component in terms of the sex and maturation effects on BRS. Evaluating the neural component of BRS in children and adolescents is difficult as most studies use invasive pharmacological techniques to quantify the individual components.[40] This is an unlikely option in children and adolescents. The findings of the present thesis demonstrate the importance of developing techniques to evaluate neural alterations during puberty, and its influence on BRS. Researchers have effectively quantified the individual components non-invasively using advanced imaging techniques and transfer function analysis.[31] Currently, our laboratory is attempting to implement similar techniques in children and adolescents. Knowledge of the specific component(s) responsible for baroreflex function will allow researchers to implement specific interventions in order to improve BRS.

Lastly, based on the results of Chapters 6 and 7, an important future direction is to further understand the importance of CS distensibility on BRS. This work is necessary in both children and adults, as little attention has been given to the CS. Evaluating CS maturation and its relationship to BRS will provide further insight into the importance of the mechanical component on BRS in children and adolescents. The finding from Chapter 7 that relative change in CS distensibility is significantly related to the relative change in BRS with orthostatic stress suggests that maturation of the CS and its relationship to BRS should be evaluated over the CCA.

8.5 Limitations

Maturation was self-reported using the criteria of Tanner. Although this has demonstrated good agreement with physician reported maturation stage using Tanner,[51] there may be bias in the self-reported stages, and difficulty in deciphering between stages.

Furthermore, the timing and tempo of maturation was not evaluated because Tanner stage was reported on one visit. Therefore, a given group can encompass individuals at the early, or late end of a given stage. Also, the studies in the current thesis did not measure sex hormone concentrations. This would provide important information on the impact of sex hormones on changes in BRS in children and adolescents. The use of sex hormones could also aid in the verification of pubertal stage. Although this would provide some insight into pubertal development, threshold sex hormone concentrations have not been linked to specific Tanner stages. Therefore, having sex hormone concentrations would not necessarily help improve pubertal staging.

The effect of sex hormones on BRS has been documented.[25-27, 52-57] Research in adults regarding the impact of sex hormones on BRS has been studied throughout phases of the menstrual cycle, with inconsistent findings.[58-61] The long-term hormonal alterations that occur during puberty and their impact on autonomic control are likely more complex than the acute changes in hormones that occur throughout the menstrual cycle. Therefore, future research is needed in this area in children and adolescents in order to fully understand maturation of autonomic function. We believe that our findings provide strong support for more detailed evaluation of the impact of sex hormones on BRS in future studies. Moreover, menstrual cycle phase was not controlled for in the present studies. Fluctuations in sex hormones throughout the menstrual cycle have been shown to influence BRS; however, this finding is inconsistent in adults and has not been examined in adolescents. A study by Hayashi et al., (2006)[61] failed to demonstrate variations in BRS throughout the menstrual cycle in young women (20 years old), while Tanaka et al., (2003)[58] demonstrated a menstrual cycle effect in older women (24 years old). Hayashi

suggested the lower concentration of estrogen in younger women might explain the inability to replicate the findings of Tanaka. This rationalization would certainly be applicable to a younger adolescent population, as well. Similar disparities exist in the literature with the effect of menstrual cycle phase on arterial distensibility in adults.[61, 62] Additionally, given greater menstrual cycle irregularity in adolescent girls and longer intermenstrual cycle length, hormone concentrations are likely to be lower and demonstrate more gradual variations. Furthermore, menstrual cycle irregularity increases the complexity of participant scheduling with no previous experimental evidence supporting its importance in adolescents. Therefore, not controlling for menstrual cycle phase in the present studies is deemed to be acceptable and not believed to have impacted the results.

The effect of time of day on BRS may also be an important limitation. It has been shown that BRS is consistently lower in the morning.[63, 64] The mechanisms involved are not fully understood; however, Taylor et al., (2011)[65] suggest this may be attributed to both mechanical and neural component alterations. BRS calculated by rising BP is reduced in the morning due to mechanical component reductions, whereas a reduction in BRS in response to decreasing BP has been attributed to the neural component. This is important to consider when evaluating BRS over repeated measurements, as well as between groups. Participants were tested at various times of the day due to participant and parent availability, and should be considered a limitation of the present studies.

A final limitation to address is the impact of physical activity and fitness as a possible confounder. Several studies in adults have shown the beneficial effects of physical activity and exercise training on BRS and arterial distensibility.[30, 41, 66] Research

regarding physical activity and fitness levels and BRS in children and adolescents has received little attention. It has been shown that physical activity and fitness are predictors of autonomic function in children and adolescents.[15, 67, 68] Adolescence is a time period of marked changes in physical activity that often results in a decline in activity levels.[69-71] It is important to note that physical activity levels have been shown to decline more rapidly in females than males.[72] Therefore, it is not likely that physical activity levels influenced the findings of decreased BRS with maturation in males and greater BRS in females post-puberty. As well, we have shown previously that peak VO_2 is not related to BRS in a sample of adolescents.[73] Further research is necessary to understand the importance of physical activity and fitness on BRS in children and adolescents.

8.6 References

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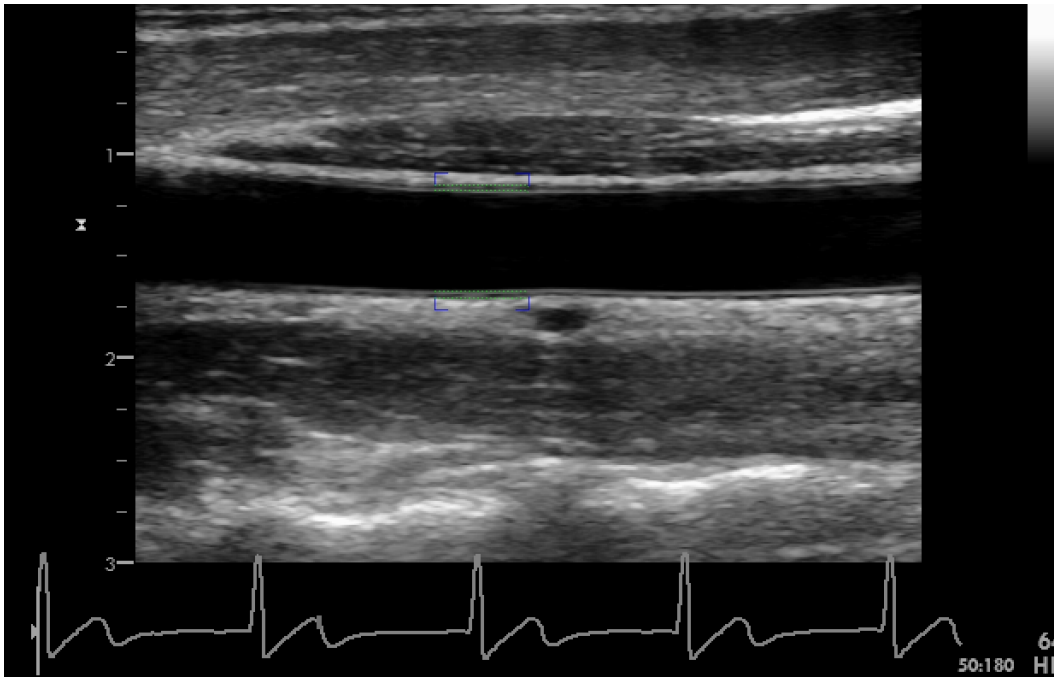
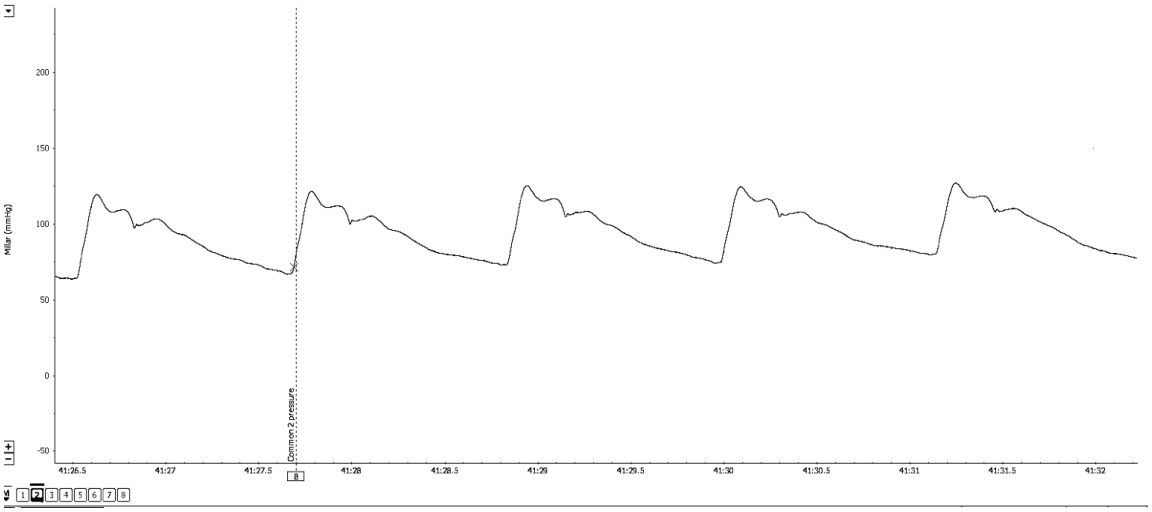
Appendix

A) Example of CCA ultrasound image and corresponding pressure waveforms for a participant.

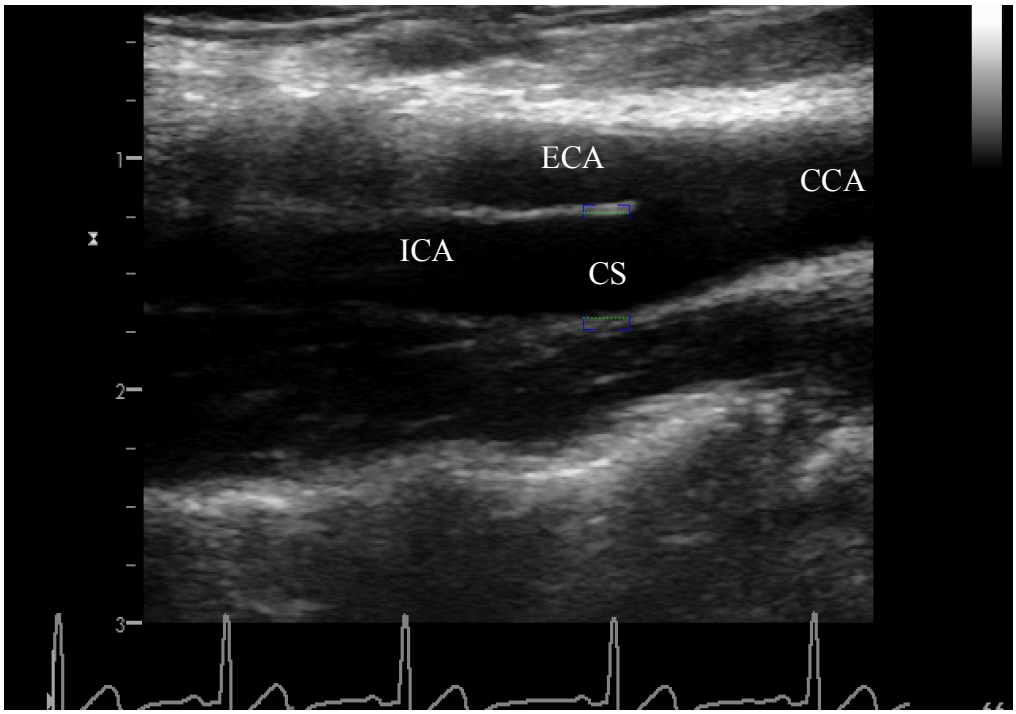


A-1) Common carotid artery 2-D B-mode image 1-2 cm proximal to the carotid bifurcation. Included is region of interest (boxed area) and diameter measurement using semi-automated edge-detection software.

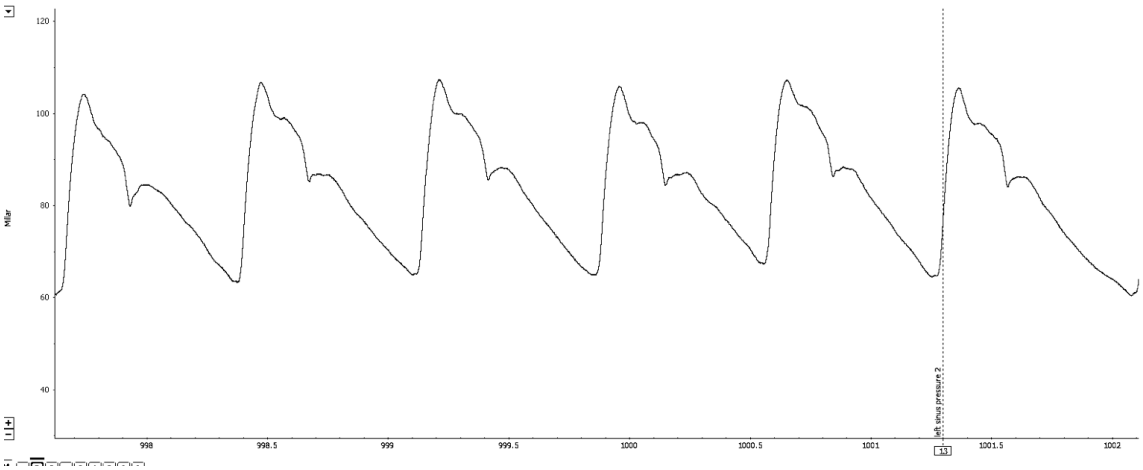


A-2) Common carotid artery pressure waveform using applanation tonometry.

B) Example of CS ultrasound image and corresponding pressure waveforms for a participant**.**

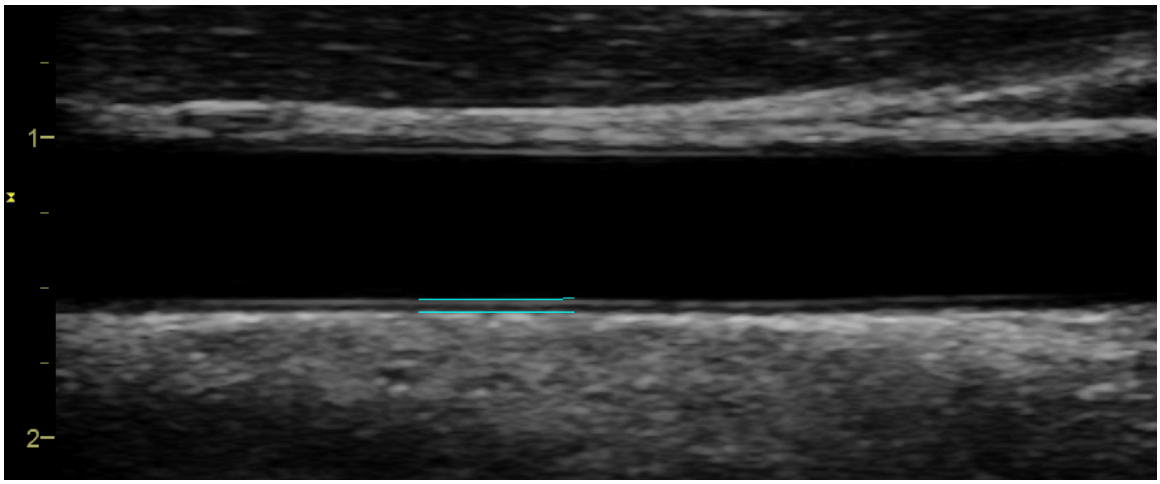


B-1) Carotid bifurcation 2-D B-mode image. ICA=internal carotid artery; ECA=external carotid artery; CS=carotid sinus; CCA=common carotid artery. Included is region of interest (boxed area) and diameter measurement using semi-automated edge-detection software.



B-2) Carotid sinus pressure waveform using applantion tonometry.

C) Example of analysis for IMT measurement.



B-mode ultrasound image of the right common carotid artery. Green lines represent measurement of intima-media thickness of the posterior wall. The top line is landmarked at the lumen-intimal border and the bottom line is landmarked at the medial-adventitial border.

D) Ethics Approval Forms

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Social Science Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	8/10/2011
PRINCIPAL INVESTIGATOR:	FALK, Bareket - PEKN
FILE:	10-260 - FALK
TYPE:	Faculty Research

TITLE: Brock Active Muscles; Muscle Activation in Children vs. Adults (Cardiovascular Parameters)

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 8/31/2012

The Brock University Social Sciences Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from 8/10/2011 to 8/31/2012.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 8/31/2012. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: [redacted]

Jan Frijters, Chair
Social Sciences Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: June 5, 2012

PRINCIPAL INVESTIGATOR: FALK, Bareket - Kinesiology

FILE: 10-260 - FALK

TYPE: Faculty Research

TITLE: Brock Active Muscles; Muscle Activation in Children vs. Adults (Cardiovascular Parameters)

ETHICS CLEARANCE GRANTED

Type of Clearance: MODIFICATION Expiry Date: 8/31/2012

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **6/5/2012** to **8/31/2012**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **8/31/2012**. Continued clearance is contingent on timely submission of reports.

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c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: 

Brian Roy, Chair
Bioscience Research Ethics Board

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Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: 8/29/2012

PRINCIPAL INVESTIGATOR: FALK, Bareket - Kinesiology

FILE: 10-260 - FALK

TYPE: Faculty Research STUDENT:
SUPERVISOR:

TITLE: Brock Active Muscles; Muscle Activation in Children vs. Adults (Cardiovascular Parameters)

ETHICS CLEARANCE GRANTED

Type of Clearance: RENEWAL Expiry Date: 8/30/2013

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **8/29/2012 to 8/30/2013**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **8/30/2013**. Continued clearance is contingent on timely submission of reports.

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c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: 

Brian Roy, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

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Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: 9/4/2013

PRINCIPAL INVESTIGATOR: FALK, Bareket - Kinesiology

FILE: 10-260 - FALK

TYPE: Faculty Research STUDENT: Daniele Chirico
SUPERVISOR: Deborah O'Leary

TITLE: Brock Active Muscles; Muscle Activation in Children vs. Adults (Cardiovascular Parameters)

ETHICS CLEARANCE GRANTED

Type of Clearance: RENEWAL Expiry Date: 8/29/2014

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **9/4/2013 to 8/29/2014**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before **8/29/2014**. Continued clearance is contingent on timely submission of reports.

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d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: 

Brian Roy, Chair
Bioscience Research Ethics Board

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